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The ectodermal signaling molecule ectodysplasin (EDA) regulates both the morphogenesis of scalp follicular units and hair growth properties

KM Huttner,[1] M Landan,[2] R Johnson,[1] K Jones,[2] A Goodwin,[2] A Jheon[2] and O Klein[2] *1 Edimer Pharmaceuticals, Inc., Cambridge, MA and 2 UCSF, San Francisco, CA*

Mutations in the EDA gene are associated with congenital hypotrichosis and premature hair thinning in males with X-linked hypohidrotic ectodermal dysplasia (XLHED). This clinical study utilized quantitative assessment technology to identify the unique hair biology associated with this developmental disorder. 14 XLHED adolescent to young adult males and 13 controls, primarily Caucasian, were enrolled in a study correlating EDA genotype and phenotype. No subject was receiving treatment for hair loss. Sequential phototrichograms from the occipital scalp were analyzed for hair and follicular unit density, hair width and growth rate, and % anagen phase. Technical staff completed photograph analysis unaware of subject diagnosis. Data available from 12 XLHED subjects and 12 controls demonstrated that EDA-deficiency was associated with a statistically-significant reduction in the scalp hair structural components including follicular units (FU)/cm2 (mean 96 vs. 152), hairs per FU (mean 1.08 vs. 1.67) and non-vellus hairs/cm2 (mean 82 vs. 219). Hair properties were affected as well with a reduction of 27% in mean width (50.1 vs. 68.4 microns), and a 34% in mean growth rate (10.5 vs. 16.0 microns/hr). Surprisingly, % anagen phase did not appear to be affected (mean 85.5 vs. 88.8%) and the vellus hair counts were similar. In the unique XLHED subject with a hypomorphic in-frame deletion, partial EDA activity was associated with an intermediate phenotype for FU, hair number and hairs/FU, but not for hair growth properties (width and growth rate). Previous human and mouse data reported that activation of the EDA signaling pathway is associated with follicle enlargement and changes in hair shape and thickness. Our findings in EDA-deficient XLHED subjects are supportive of a role for EDA both in hair appendage development and in ongoing hair growth, possibly contributing to population variation in hair form and hair thinning.

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L. Digitata influences expression of genes related to vasculature and may therefore be beneficial for improving the appearance of cellulite

R Gopaul, DG Kern and HE Knaggs *Center for Anti-aging Research, Nu Skin Enterprise, Provo, UT*

Cellulite is a result of fat deposits often occurring on the hips, buttocks and thighs creating a dimpling, orange-peel appearance on skin. Vasoconstriction often accompanies the formation of cellulite but it is not known whether this results from the increasing adipocyte size or is causative. Nevertheless, vasoconstriction results in reduced blood flow reducing nutrient supply to upper areas of skin, weakening the skin's connective tissues and possibly contributing to the dimpling effect seen in persons with cellulite. Therefore, one way to prevent or improve cellulite is by decreasing vasoconstriction and increasing vasodilation. In this study, two concentrations of Laminaria Digitata (L .Digitata) Extract were tested on human full-thickness 3D epidermal skin equivalents and primary human adipocytes. Gene expression was measured by quantitative PCR using custom TaqMan Low Density Arrays (TLDA) after 24 hours of incubation on both tissue types. Compared to untreated control, L .Digitata upregulated genes related to vasodilation on epidermal skin equivalents and adipocytes. Compared to untreated control, L. Digitata also downregulated genes related to vasoconstriction on adipocytes. Genes that were upregulated include H1FA, VEGFA and HP. Genes that were downregulated include ADRAIA, ADRAID, H1FA and HP. The findings from this study indicate a possible role of L. Digitata in improving the appearance of cellulite by producing molecules that may cause an impact in the vasculature.

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L. Digitata influences gene expression relating to adipogenesis and lipolysis and thus may be used as an active ingredient for addressing cellulite

R Gopaul, DG Kern and HE Knaggs *Center for Anti-aging Research, Nu Skin Enterprise, Provo, UT*

One of the main differences in body skin versus facial skin is localized fat deposition and accumulation under body skin. Two key processes responsible for fat deposition are adipogenesis and lipolysis. Adipogenesis is the differentiation of preadipocytes into adipocytes . Lipolysis is the breakdown of lipids via hydroxylation of triglycerides into free fatty acids. An increase in adipogenesis and decrease in lipolysis can lead to localized fat deposition and accumulation on certain areas of the body, potentially resulting in an orange-peel effect. Localized fat deposition may also cause the extracellular matrix (ECM) to be compromised making the skin appear loose and wrinkled in some individuals. In this study, Laminaria Digitata (L .Digitata) Extract was evaluated for its effects on lipolysis and adipogenesis and subsequent influences on the appearance of body skin. Two levels of L .Digitata were tested on primary human adipocytes and human full-thickness 3D epidermal skin equivalents. Gene expression was measured by quantitative PCR using custom TaqMan Low Density Arrays (TLDA) after 24 hours of incubation on both tissue types. Compared to untreated control, L .Digitata regulated genes related to activation of lipolysis and reduction in adipogenesis on adipocyte cells. Genes known to improve the integrity of the extracellular matrix were also regulated on human equivalent epidermal skin equivalents. Genes that were regulated related to lipolysis and adipogenesis include, but not limited to ASIP, PDE5A, KFL6 and ADRP. Genes that were regulated in support of ECM integrity include, but not limited to COL4A1, CTGF, DSG2 and TIMP1. Based on this data, it can be hypothesized that L .Digitata may play a role in decreasing adipogenesis while increasing lipolysis. This effect may also have a consequent benefit in improving the ECM of the skin.

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Identification of genes promoting exceptional skin youthfulness

AS Chang,[1] G Atzmon,[2] A Bergman,[2] HY Chang[1] and N Barzilai[2] *1 Dermatology, Stanford University School of Medicine, Redwood City, CA and 2 Albert Einstein College of Medicine, Bronx, NY*

While many genetic and environmental factors are known to promote skin aging, the genes controlling exceptional skin youthfulness have not been identified. Anecdotally, skin aging is not a universal fate, as some centenarians and their offspring possess skin that appears decades younger than their chronological age. To identify genes which promote skin youthfulness (SY) as well as identify possible co-morbidities which might associate with this phenotype, 428 individuals of Ashkenazi-Jewish descent from the LonGenity Database were subjected to genome-wide association study (GWAS) based on skin aging parameters assessed using a newly validated and reproducible facial skin aging scale applicable to this population with advanced age. In addition, prevalence rates of co-morbid medical conditions in those with and without SY were compared. We identified 11 single-nucleotide polymorphisms (SNPs) which are candidate SY genes at a significance level of $p<10\text{-}8$. The validation group consisted of 436 additional individuals from this same database and resulted in the validation of two genes, one encoding an ion channel ($p=0.023$) and one encoding a transcription factor ($p=0.045$). Both genes are expressed in skin. Multivariate analysis of available co-morbid medical conditions in 452 subjects, adjusted for age, BMI, IGF1, HDL and TSH levels showed that decreased TSH or decreased HDL levels was significantly associated with a higher likelihood of SY phenotype ($p=0.033$ for both). These genes and co-morbidities merit further investigation into their biologic significance in contributing to the SY phenotype. Additional validation groups will be needed to confirm these findings and better define the relevance of these findings to non-Jewish populations.

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Rejuvenation of gene expression patterns in aged human skin with broadband light treatment

AS Chang,[1] P Bitter,[2] K Qu[1] and HY Chang[1] *1 Dermatology, Stanford University School of Medicine, Redwood City, CA and 2 Advanced Aesthetic Dermatology, Los Gatos, CA*

Aging is associated with large-scale changes in gene expression; how such change may be modulated for healthful benefits in humans is not clear. One of the most popular technologies in use for cosmesis of skin aging is broadband light (BBL) with over $215 million dollars spent in the United States in 2009 on this procedure. While the visible effect is to "rejuvenate" the skin, the molecular changes are unknown. Here we explore whether BBL truly "rejuvenates" skin on a molecular level, or simply creates a wounding effect. RNA sequencing of arm skin biopsies from 5 female volunteers age>50 years with moderate to severe photodamage after 3 treatments of BBL was performed. These sequences were compared to those from untreated ajacent skin as well as untreated arm skin from 5 females <30 years of age. We found systematic changes in RNA expression with old untreated and old BBL treated groups compared to young group. 305 genes qualified as "rejuvenated" genes with $p<0.05$ and accounting for multiple comparisons. These included: ZMPSTE24, a metalloproteinase that processes lamin A, a gene defective in progeria; IGFR1, linked to longevity in mice; EIF4G1 and EIF4EBP1, which are associated with lifespan in C. elegans; RING1 and MOV10, both in the polycomb pathway that controls lifespan of human fibroblasts. Of the "rejuvenated" genes, 42 were long non-coding RNAs (lncRNAs). These included: PVT1, most proximate to MYC gene; loc146880, most proximate to senescence-linked gene SMURF2; IGF2AS, most proximate to IGF2, which is associated with age-related degerative diseases. No changes in RNA 3′ end processing were found with aging. These data suggest that BBL treatment can restore gene expression pattern of aged skin to resemble young skin. In addition, this study identifies a number of lncRNAs which are "rejuvenated" and whose future study may lead to new insights into the human skin aging process.

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Genome wide association analysis of psoriatic arthritis

RP Nair,[1] LC Tsoi,[2] V Chandran,[3] PE Stuart,[1] T Tejasvi,[1] JJ Voorhees,[1] GR Abecasis,[2] P Rahman,[4] JT Elder[1] and D Gladman[3] *1 Dermatology, University of Michigan, Ann Arbor, MI, 2 Biostatistics, University of Michigan, Ann Arbor, MI, 3 Toronto Western Hospital, Toronto, ON, Canada and 4 Memorial University, St John's, NF, Canada*

Psoriasis (PsC) is an autoimmune inflammatory skin disease with at least 18 well-established genetic susceptibility loci. About 15-25% of PsC patients also develop psoriatic arthritis (PsA). Since nearly all PsA patients also have PsC, it is difficult to establish presence of distinct genetic loci involved in development of arthritis symptoms. Understanding of the PsA predisposing loci has predictive value in designing therapies that can prevent PsA development. To date, all known PsA loci (TRAF3IP2, REL, TNIP1, IL23A, IL12B, MHC) have also been implicated in PsC. In order to delineate the genetic susceptibility underlying PsA from PsC, we performed a genome-wide association study of European-origin PsA patients and controls and compared the results to previous genotyping of independent PsC cases and controls. The study sample consisting of 1526 PsA patients and 1508 unaffected controls were genotyped on the Illumina Omni-1 Quad chip set (1,048,713 SNPs). After quality control, 1442 cases and 1433 controls were analyzed for association of 814,114 SNPs using a variance component approach. Four loci (TNIP1, IL12B, MHC, and TYK2) yielded genome-wide significance ($p < 5 \times 10^{-8}$), of which TYK2, a known PsC locus, is identified for the first time for PsA. One known PsA locus (TRAF3IP2), and three novel loci (at chromosomes 2q35, 10q21.3 and 14q22.1) showed potential evidence of association with $p < 1 \times 10^{-5}$. For the MHC and IL12B, PsA signals differed from PsC signals, suggesting the presence of arthritis loci close to PsC loci or distinct arthritis-predisposing alleles at the PsC loci. Thus our analysis identified one new genome-wide significant PsA locus and three potential loci that require further exploration using an expanded sample size.

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Two- and three-dimensional human keratinocyte cell culture: A transcriptomic comparison of biological function modulation

AP Azambuja,[1] M Lorencini[1] and N Remoué[2] *1 Science and Technology Ideas and Concepts, Natura, Cajamar, Brazil and 2 Technical Center - Paris, Natura, Paris, France*

Cell culture models have been considered important tools in cell and molecular research based on the availability of clear and direct comparison between treatments in association with the low cost and high speed testing. Although, little is known regarding the molecular profile or biological functions activated in cultured normal keratinocytes. Here we describe, using transcriptomic characterization of cultured human normal keratinocytes, that monolayer (2D) and stratified cell (3D) culture models can induce distinct molecular profiles stimulating relevant divergences in common cellular functions (|fold change|≥3, p-value≤0,001 e q-value≤0,001). We describe that while 2D cultures clearly activate genes related to tissue adhesion and growth, lipid metabolism and cell differentiation are the main functions upregulated in 3D models. Also, it was observed the activation of skin diseases markers as PSORS1, DEFB103B and CYP4F22 in the 3D model. Hitherto, we show that both culture models can suppress donor molecular backgrounds, such as age, leading the discussion on the advantages and limitations of 2D and 3D keratinocyte culture models in dermatological studies. Together these results raise the debate on the need of careful evaluation of culture models application on molecular and cellular skin research.

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Genetic variants in genes of the collagen and melanin metabolism influence extrinsic skin aging of the face

A Vierkötter,[1] E Rosenbaum,[1] T Schikowski,[3] D Sugiri,[1] M Matsui,[2] U Krämer[1] and J Krutmann[1] *1 IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany, 2 Estee Lauder Inc., Melville, NY and 3 Swiss Tropical and Public Health Institute, Basel, Switzerland*

Extrinsic skin aging results from chronic exposure to environmental factors including ultraviolet radiation, tobacco smoke, and, as recently shown, traffic-related air pollution. Molecular mechanisms for extrinsic skin ageing include increased degradation of dermal collagen fibres as a major cause for the manifestation of wrinkles, whereas a deregulated melanin metabolism contributes to the development of pigment irregularities. However little is currently known about the relevance of the individual genetic make-up for extrinsic skin aging. We have therefore asked whether genetic variants in genes, which are functionally involved in the regulation of the skin's collagen and melanin metabolism, might influence the clinical manifestation of extrinsic skin aging. In our SALIA cohort we evaluated skin ageing symptoms in N=377 elderly women (mean age: 74 years) by SCINEXA, a validated clinical score. The adjusted association between the severity of wrinkles and pigment irregularities with different genetic variants of the collagen and melanin metabolism was analyzed by linear regression. Regarding genetic variants in the collagen metabolism we found that the single nucleotide polymorphism (SNP) rs1799750 in the matrix-metalloproteinase-1 gene was significantly associated with the formation of coarse wrinkles. In addition, regarding genetic variants in the melanin metabolism we found various SNPs in the genes encoding for melanocortin-1-receptor(M1CR), tyrosinase, tyrosinase-related-protein-1(TYRP1), two-pore-segment-channel-2(TPCN2), agouti-signalling-protein(ASIP) and solute-carrier-family-45-member-2(SLC45A2) to be significantly associated with pigment irregularities. These results indicate that genetic variants in the collagen and melanin metabolism influence extrinsic skin aging of the face and corroborate our assumption that the individual genetic make-up is critically involved in this process.

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CSA and CSB proteins localize in a large multiproteincomplex involved in repair and transcription of mitochondrial DNA: Involvement for neurodegeneration

Y Kamenisch, S Giovannini, N Düzenli, M Röcken and M Berneburg *Dermatology, Eberhard Karls University Tuebingen, Tuebingen, Germany*

Cockayne syndrome is a rare progeroid disorder, characterized by sun sensitivity of the skin, premature loss of subcutaneous fat and neurodegeneration. CS is caused by dysfunctional CSA or CSB proteins, which are involved in transcription and repair of nuclear DNA. We have previously shown, that CSA and CSB proteins are also associated to the mitochondrial DNA protein complex (nucleoid) and protect from stress or aging induced accumulation of mtDNA mutations and protect from aging associated loss of subcutaneous fat tissue. It is known, that mutations of mitochondrial DNA can cause aging symptoms, but less is known about the proteins which are associated to the mitochondrial DNA. The nucleoid is the structure in the mitochondria, where the mitochondrial DNA and several protein complexes are located. In order to further investigate the role of mitochondrial CSA and CSB proteins we developed a new assay to screen mitochondrial nucleoids for protein interaction partners. After isolation of mitochondria and subsequent precipitation of nucleoid complexes the different nucleoid complexes were separated and visualized by blue native gel electrophoresis and proteins were detected by an additional second dimension SDS gel separation followed by western blot analysis. We found that CSA and CSB containing protein complexes also harbor the proteins, which are essential for mitochondrial transcription: mtRNA polymerase and mitochondrial transcription factor A (mtTFA). In addition we found a protein involved in the pathogenesis of Parkinson disease also in the CSA and CSB associated mtDNA protein complex. These findings point to a role of mitochondrial CSA and CSB in mitochondrial transcription, and provide a potential mechanism for the pathogenesis of the neurodegenerative symptoms of the progeroid Cockayne syndrome.

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Recombinant filaggrin successfully internalized and processed in epidermal cells

T Stout, T McFarland and B Appukuttan *Oregon Health & Science University, Portland, OR*

This study was designed to engineer a functional filaggrin monomer linked to a cell penetrating peptide (CPP) and test the ability of this peptide to penetrate epidermal tissue as a therapeutic strategy for genetically determined atopic dermatitis (AD). A single repeat of the murine filaggrin gene (Flg) was amplified using primers including a 3′ CPP-motif (RMR) and cloned into a bacterial expression system for protein production (GenScript Inc.). Purified filaggrin-RMR (FLG-RMR) was applied *in vitro* to HEK 293 cells and a reconstructed human epidermis (RHE) tissue model (EpiDerm, MatTek Corp.) and *in vivo* to filaggrin-deficient flaky tail (ft/ft) mice skin. Immunohistochemical and western blot techniques were employed to determine RMR-dependent cellular internalization of FLG-RMR and appropriate cellular processing. Immunochemistry demonstrated RMR-dependent cellular uptake of FLG-RMR in a dose- and time-dependent manner in HEK cells. Filaggrin lacking RMR was unable to penetrate the cells. Immunohistochemical staining of the RHE model identified penetration of FLG-RMR to the stratum granulosum, the epidermal layer at which filaggrin deficiency is thought to be pathologically relevant. Staining of flaky tail mouse skin supported the *in vitro* results with the identification of FLG-RMR uptake after application. Western blotting of flaky tail epidermal tissue after FLG-RMR application demonstrated the breakdown of FLG-RMR into appropriately sized monomers consistent with the natural processing of native filaggrin, indicating cellular penetration of FLG-RMR and subsequent processing. These results suggest that topically applied RMR-linked filaggrin monomers are able to penetrate epidermal tissue, be internalized into the appropriate cell type and be processed to a size similar to wild-type functional barrier peptides. Therefore, topical application of recombinant filaggrin may restore necessary barrier function and prove therapeutic for patients with AD. Methods of RMR-mediated topical protein introduction may also be extended to the treatment of other skin disorders involving protein deficiency.

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Circadian genes are expressed in human sebocytes and regulate sebaceous lipid production

S Berkovitz,[1] M Qin,[1] M Kim,[1,2] G Agak,[1] DM Thiboutot[3] and J Kim[1,4] *1 Division of Dermatology, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA, 2 Department of Dermatology, Dankook Medical College, Cheonan, Republic of Korea, 3 Department of Dermatology, Penn State University College of Medicine, Hershey, PA and 4 Department of Medicine, Greater Los Angeles Healthcare Service Veterans Affairs, Los Angeles, CA*

Many skin functions follow diurnal oscillations according to an internal timing system, the circadian clock. The amount of facial sebum excreted has been shown to exhibit daily oscillation with maximum at noon and minimum in the early morning. A circadian rhythm has been described *in vitro* in keratinocytes and fibroblasts, but not in sebocytes, cells central to acne pathogenesis. In this study, we aim to characterize the circadian regulation of sebocytes using the immortalized human sebocyte cell line (SEB-1). Using reverse transcription polymerase chain reaction (RT-PCR), we demonstrate the expression of the clock gene family in sebocytes. With quantitative RT-PCR, we show that clock gene expression oscillates over time. Small interfering RNA knock-down of a core clock gene, brain and muscle Arnt-like protein-1 *(Bmal1)*, reveals a marked reduction in sebaceous lipids, as visualized by Nile Red stain. Flow cytometry confirms this observation, showing a histogram shift to the left in the *Bmal1* knockout cells indicating reduced sebaceous lipid content. Quantitative RT-PCR of the *Bmal1* knockout cells shows significantly reduced expression of lipid-associated genes including farnesyl-diphosphate farnesyltransferase (*Fdft1*), or squalene synthase, and melanocortin-5 receptor (*Mc5r*), a marker of differentiated sebocytes. This down-regulation of lipid-associated genes persists even with *Propionibacterium acnes* infection, which is expected to increase sebaceous lipids. In conclusion, we are the first to report the expression of circadian genes in human sebocytes and the function of the clock gene *Bmal1* in regulating sebum production. Elucidating the circadian rhythm's role in skin may help us better understand the function of sebocytes and optimize treatment for acne and other skin diseases.

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Role of Jun proteins in epidermal growth regulation

SX Zhang, JY Jin and JY Zhang *Dermatology, Duke University, Durham, NC*

AP-1 transcription factors play important roles in tissue homeostasis of various organs. Our previous studies have demonstrated that c-Jun and JunB display opposite effects on epidermal growth and neoplasia with JunB being the negative regulator. The goal of this study is to understand the molecular mechanisms mediating the subunit specific functions. We first demonstrated that c-Jun was activated in human keratinocytes within 1h following EGF-treatment, whereas JunB was activated in response to Ca++ at 24h and 48h time-points. In addition, our preliminary CHIP-PCR analysis revealed that both c-Jun and JunB interacted with the gene promoters of filaggrin and involucrin following Ca++-treatment. In contrast, only c-Jun displayed interaction with EGFR promoter in response to EGF-treatment. Further growth analysis with synthetically tethered Jun-Fos dimers revealed that exogenous expression of JunB-Fra1 and JunB-c-Fos dimers decreased cell growth of primary human keratinocytes, which was associated with increased cell senescence and decreased S-phase cell cycle progression. Interestingly, c-Jun-Fra1 but not c-Jun-c-Fos also induced a reduction in S-phase progression. Additionally, the protein dimers altered the intensity and staining pattern of mitochondria. Taken together, these findings implicate that Jun-proteins are differentially involved in regulating epidermal cell growth, differentiation and metabolism via dimerization with Fos-family subunits. Currently, our efforts are focused on identifying other Jun protein targets at a genome scale and investigating the role of Jun-Fos dimers in 3-dimentional skin culture and skin grafting models.

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Age-dependent change of caveolin-1 expression in the skin: A marker for skin aging

S Lee, J Lee and J Choi *Department of Dermatology, Chonnam National University Medical School, Gwangju, Republic of Korea*

There are a variety of biomarkers for chronological aging in the body, and caveolin was suggestive to be implicated in the aging process in the skin. This study was aimed to evaluate the modulation of caveolin-1 (Cav-1) in relation with collagen levels in the skin. Age-dependent change in the expression levels of Cav-1 and collagens I and III (COL I/III) were compared between the young and aged skin of mouse and human *in vivo*, and between the young and senescent human dermal fibroblasts (HDF) *in vitro*. In RT-PCR and Western blot analysis, mRNA and protein levels of Cav-1 were up-regulated, but those of COL I/III were down-regulated, from the aged skin of human and mouse, and also in the senescent HDF. Immunohistochemical staining revealed that Cav-1 was up-regulated both in the epidermis and dermis of the aged human skin. Immunocytochemical staining showed that Cav-1-positive signals were markedly increased in the cytoplasm of senescent HDF. The reciprocal negative correlation between Cav-1 and COL I/III was confirmed from our results that COL I/III were markedly up-regulated in Cav-1 siRNA transfected DFs or Cav-1(-/-) knock-out mice. Collectively, Cav-1 is up-regulated in relation with decreased COL I/III expression in the aged skin. Our results suggest us that Cav-1 can be a novel biomarker for skin aging.

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Functional analysis of candidate genes identified by genomewide association study (GWAS) of infantile hemangiomas (IH)

EM Kwon,[1] DH Siegel,[1,2] U Broeckel,[2,4] PE North,[3] E Worthey,[2,4] YE Chiu[1,2] and BA Drolet[1,2] *1 Dermatology, Medical College of Wisconsin, Milwaukee, WI, 2 Pediatrics, Medical College of Wisconsin, Milwaukee, WI, 3 Pathology, Medical College of Wisconsin, Milwaukee, WI and 4 Human and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI*

Infantile hemangiomas (IH) are benign tumors comprised of proliferating endothelial-like cells. Our current hypothesis predicts that both genetic and environmental factors contribute to the pathogenesis of IH. Preliminary results from our genomewide association study (GWAS) using the Affymetrix 6.0 chip to assay 906,600 single nucleotide polymorphisms (SNPs) in 224 IH family trios identified 36 SNPs that met genome-wide significance filters ($p<10^{-8}$). Candidate genes near identified SNPs were prioritized based on *in silico* functional characterization and pathway and binding interactions. Prioritization was assigned to NOSTRIN (rs7588452, $p=5.85x10^{-8}$), PELP1 (rs9903855, $p=5.73x10^{-7}$), and MLL (rs9332754, $p=6.11x10^{-6}$). In order to determine functional significance to the leading candidate genes, we evaluated the expression patterns of their gene products in surgically excised IH tissue from study participants. NOSTRIN (eNOS trafficking inducer protein) was identified as a key candidate based on participation in the eNOS/hypoxia pathways, interaction with previously described IH candidate genes, and association with known clinical risk factors including pregnancy-induced hypertension and preeclampsia. NOSTRIN staining utilizing a polyclonal antibody (ProteinTech) was applied to 9 formalin-fixed, paraffin-embedded IH tissue samples by immunohistochemistry (IHC). Staining of lesional capillary endothelial cells was robust in 9/9 of the study IH specimens, whereas adjacent arteries showed much less intense staining. Positive control samples (placenta and renal cortex) were highlighted by NOSTRIN IHC. Negative controls were provided internally and externally by smooth muscle cells, pericytes and other stromal elements. Localization of the NOSTRIN protein to IH endothelial cells supports GWAS data, implicating NOSTRIN in IH pathogenesis.

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Progressive ectodermal phenotype of costello syndrome

EM Kwon,[1] AH Rosen,[1] AS Paller[3,4] and DH Siegel[1,2] *1 Dermatology, Medical College of Wisconsin, Milwaukee, WI, 2 Pediatrics, Medical College of Wisconsin, Milwaukee, WI, 3 Dermatology, Northwestern University Feinberg School of Medicine, Chicago, IL and 4 Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL*

The RASopathies encompass a group of medical genetic syndromes caused by germline mutations in various genes encoding proteins involved in the Ras/mitogen-activated protein kinase (MAPK) pathway. Costello syndrome (CS) is a rare RASopathy caused by activating mutations in the HRAS gene, an important regulator in signal transduction. The dermatological phenotype of CS includes cutaneous papillomas, palmoplantar keratoderma (PPK), acanthosis nigricans (AN), and pachydermatoglyphia, features shared among cutaneous paraneoplastic syndromes. In this study, we specifically focused on the correlation of the keratotic features of CS with the age of mutation positive individuals. Twenty-six individuals with CS and confirmed HRAS mutations participated in the study. Ages ranged from 5.5 months to 34 years. Dermatologic surveys were designed and completed by the authors upon interview with parents and physical examination of individuals with CS at the Costello Syndrome Family Network conference in 2011. Scores were assigned by the authors to each of the following five categories: cutaneous papillomas, PPK, pachydermatoglyphia, AN, and keratosis pilaris based on the presence and severity of each phenotype. Age was significantly correlated with papillomas ($r=0.409$, $p=0.038$), pachydermatoglyphia ($r=0.563$, $p=0.003$), and AN ($r=0.466$, $p=0.016$). Our current hypothesis predicts that dysregulation of the RAS signaling pathway contributes to the development of the keratotic features of CS given its progressive ectodermal phenotype and shared features with cutaneous paraneoplastic syndromes. Information on the relationship between the cutaneous features of CS and Ras/MAPK signaling would not only provide further insight into the pathway, but would also have implications for potential therapies.

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A missense mutation within the helix initiation motif of the keratin 71 (KRT71) gene underlies autosomal dominant woolly hair/hypotrichosis

A Fujimoto,[1,2] A Inoue,[3] M Ohyama,[4] H Fujikawa,[1,2] M Farooq,[1] R Ehama,[5] J Nakanishi,[5] M Hagihara,[5] T Iwabuchi,[5] J Aoki,[3] M Ito[2] and Y Shimomura[1] *1 Laboratory of Genetic Skin Diseases, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, 2 Division of Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, 3 Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan, 4 Department of Dermatology, Keio University School of Medicine, Tokyo, Japan and 5 Shiseido Research Center, Yokohama, Japan*

Woolly hair (WH) is a hair shaft anomaly characterized by tightly curled hair, which can frequently be associated with hypotrichosis. Non-syndromic forms of WH can show either autosomal dominant (ADWH) or recessive (ARWH) inheritance pattern. It has recently been shown that ARWH is caused by mutations in either LPAR6 or lipase H (LIPH) genes. More recently, mutations in keratin 74 (KRT74) gene have been reported to underlie ADWH. Importantly, all of these genes are abundantly expressed in the inner root sheath (IRS) of the human hair follicles. Besides these findings, molecular mechanisms underlying hereditary WH have not fully been disclosed. In this study, we identified a Japanese family with ADWH with hypotrichosis. After exclusion of known candidate genes, we discovered a heterozygous missense mutation c.422T>G (p.Phe141Cys) within the helix initiation motif of the IRS-specific keratin 71 (KRT71) gene in the affected family members. We demonstrated that the mutant keratin 71 (K71) protein led to disruption of keratin intermediate filament formation in cultured cells. To our knowledge, this is the first KRT71 mutation associated with a hereditary hair disorder in humans. Our findings further underscore the crucial role of the IRS-specific keratins in hair follicle development and hair growth in humans.

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***In vitro* models: A new approach on development of cellular models**

JC Lago,[1] R Vidal[2] and M Lorencini[1] *1 Natura Innovation and Technology of Products Ltda, Cajamar, Brazil and 2 National Biosciences Laboratory – LNBio, Campinas, Brazil*

Since the end of animal tests for cosmetic companies was determined, different *in vitro* models have been development to understand the biology of skin and how it can answer to pharmaceutical and cosmetics products. The skin is one of the most complex tissues of our body, with lots of interactions. To better understand the influence of these interactions and the cell age on cellular models, a transcriptome study with 32.000 genes was realized, comparing 3D and monolayer fibroblast cell culture using two different age's group. The results suggest that there is an influence on cell age, for mRNA expression, when the same model was considered. A similar result was found when we considered different models for cell on the same age group. Regarding these data, we suggest new considerations for choosing the cells on developing new cellular models.

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The vascular genetics translational research and gene discovery program

DH Siegel,[1,2] E Worthey,[2,3] EM Kwon,[1] YE Chiu,[1,2] U Broeckel[2,3] and BA Drolet[1,2] *1 Dermatology, Medical College of Wisconsin, Milwaukee, WI, 2 Pediatrics, Medical College of Wisconsin, Milwaukee, WI and 3 Human and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI*

The Vascular Genetics Translational Research and Gene Discovery Program at Medical College of Wisconsin (MCW) has been established to develop the infrastructure for a multidisciplinary effort to rapidly discover disease-causing mutations in individuals with rare developmental vascular disorders by utilizing next-generation sequencing and genomics analysis. This research center houses several national rare vascular anomalies registries. Patients with vascular anomaly syndromes are identified through the Center for Vascular Anomalies and Tumors Translational Research at Children's Hospital of Wisconsin and carefully phenotyped for prioritization for genomic analysis. Through the Human and Molecular Genetics Center at MCW, we have access not only to next-generation sequencing systems including a Roche 454, multiple illumina HiSeqs systems and PacBio, but also to significant computational resources including a host of analysis servers and a 200T storage pool. The HMGC also provides us with centralized support staff to maintain these large systems. We are developing a vascular specific bioinformatics program to rapidly analyze and prioritize the large amounts of data generated by sequencing through recruitment of a team of programmers and analysts with expertise in sequence analysis, bioinformatics and genomics. We are building systems to store, integrate, analyze, and visualize these large datasets. Family trios are being sequenced to enable identification of de novo mutations and assist in differentiation between benign polymorphisms and disease associated mutations. As many of these syndromes may be due to somatic mosaicism, strategies are being implemented to detect mosaicism through exome sequencing. In addition we are beginning to develop a vascular disorder disease portal, where we will collate and curate genetic and phenotype data from a variety of sources into one easy to use system.

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Dissection of Dlx3 function in ectodermal appendage development: Impacts of mesenchymal and epithelial-specific deletions on tooth development

O Duverger,[1] A Zah,[1] J Isaac,[1] H Sun,[2] JB Lian,[3] A Berdal,[4] J Hwang[1] and MI Morasso[1] *1 Developmental Skin Biology Section, NIAMS/NIH, Bethesda, MD, 2 Biodata Mining and Discovery Section, NIAMS/NIH, Bethesda, MD, 3 Departments of Cell Biology and Orthopedic Surgery, University of Massachusetts Medical School, Worcester, MA and 4 Laboratory of Molecular Oral Physiopathology, INSERM UMRS 872, Paris, France*

Dlx3 homeodomain transcription factor is involved in ectodermal appendage development and mutations in human DLX3 lead to Tricho-Dento-Osseous (TDO) syndrome. Patients with TDO syndrome have kinky hair and major tooth defects including taurodontism (enlarged pulp chamber) and enamel hypoplasia. Dlx3 is expressed in both the dental epithelium (including enamel-producing ameloblasts) and the dental mesenchyme (including dentin-producing odontoblasts) during tooth development, and both tooth compartments are affected in TDO patients. Thus far, the normal function of Dlx3 in tooth development is undocumented. To address the function of Dlx3 in the two compartments of the developing tooth, we performed conditional deletion of Dlx3 in the dental mesenchyme using Wnt1-cre mice and in the dental epithelium using K14-cre mice. Mesenchymal deletion of Dlx3 leads to the formation of defective teeth with hypoplastic dentin, resulting in enlarged pulp chambers and short roots. On the other hand, epithelial deletion results in the formation of hypoplastic and hypomineralized enamel. Taking advantage of these two animal models, we used microarray and ChIP-seq analysis to identify downstream targets of Dlx3 in odontoblasts and ameloblasts. This dual approach of Dlx3 function in tooth development sheds light on the pathways regulated by Dlx3 in this ectodermal appendage and gives new clues for investigating TDO syndrome.

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Therapies for dystrophic epidermolysis bullosa require close evaluation for development of antibodies to type VII collagen

ES Gorell, N Nguyen, Z Siprashvili, P Marinkovich and A Lane *Dermatology, Stanford School of Medicine, Stanford, CA*

Dystrophic epidermolysis bullosa (DEB) is a genetic blistering skin disease caused by a mutation in the COL7A1 gene encoding type VII collagen (C7). The amino terminal non-collagenous domain (NC1) is the most antigenic region of C7 and is pathogenic in epidermolysis bullosa acquisita (EBA). Therapeutic DEB correction will require immunologic exposure to C7 which could cause EBA if the newly expressed NC1 is recognized as foreign. Western blot analysis of cultured keratinocytes is the most sensitive test for NC1 expression and it is expected that DEB subjects already expressing NC1 are less likely to develop EBA when exposed to full length C7. We investigated if genetic analysis can predict NC1 expression and the potential to develop antibodies to NC1. We hypothesized that subjects with two trans loss-of-function mutations in exons 1-28 will lack NC1. We investigated a correlation between subjects who had received allografts containing C7, NC1 production, and C7 antibodies. Cultured keratinocytes were used for NC1 Western blot analysis. Blood was obtained for genetic testing and ELISA for IgG C7 antibodies. NC1 Western blot expression correlated with genetic testing for 2/2 NC1[-] samples and 9/9 NC1[+] samples. Immunofluorescence (IF) skin biopsies evaluated with LH7.2 monoclonal antibody were not a reliable indicator for NC1 expression for 6/11 subjects: 2 NC1[+] = [-] IF, 1 NC1[-] = [±] IF, 3 (with confirmed DEB) = [+] IF. IF predicted NC1 for 5/11: 1 NC1[-] = [-] IF, 4 NC1[+] = [±] IF. 7 subjects previously had allografts containing C7; 1 (NC1[+]) had C7 antibodies. 2 NC1[+] subjects who had not received grafts had C7 antibodies. Genetic testing correlates with Western blot assays for NC1 status and may be a useful tool to predict NC1 expression for those unable to perform Western analysis. We were unable to find a compelling association between NC1, previous C7 allograft therapy, and antibody production. EB subjects treated with C7-containing products should be tested for C7 antibodies and possible EBA development.

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Digenic inheritance in epidermolysis bullosa simplex

G Padalon-Brauch,[1] D Ben Amitai,[2] O Sarig,[1] E Sprecher[1] and Y Mashiach[1] *1 Tel Aviv Medical Center, Tel Aviv, Israel and 2 Schneider Children's Hospital, Petach Tikvah, Israel*

Epidermolysis Bullosa (EB) encompasses a heterogeneous group of hereditary disorders characterized by blistering of the skin upon exposure to mechanical stress. EB simplex (EBS) is the most common form of EB and has been associated with mutations in at least 5 distinct genes. Most EBS cases result from mutations in KRT5 and KRT14, encoding the two major basal cytokeratins, and are inherited in an autosomal dominant fashion. Recessive inheritance of KRT14 mutations has however rarely been described. In this study, we report the first instance of digenic inheritance in EBS. We ascertained a 2 year-old boy of Jewish Ashkenazi origin, who presented with widespread congenital skin blistering, mostly during the summer months. His mother as well as maternal grand-father reported a similar but significantly milder phenotype, exclusively located to the soles. In an attempt to explain the striking phenotypic discrepancy between the proband and other affected members of his family, we screened all family members for mutations in KRT5 and KRT14. The proband and his asymptomatic father were found to carry a recurrent heterozygous mutation in KRT14, p.R388H. This mutation has previously been associated with recessive inheritance of EBS either in an homozygous or compound heterozygous state. In addition, the proband was found to carry a novel heterozygous mutation in KRT5, p.I183T, affecting a highly conserved amino acid residue. The mildly affected mother and maternal grand-father of the proband also carried the same mutation in an heterozygous state. Using a PCR-RFLP assay, we excluded p.I183T from a panel of 200 population-matched control individuals. In conclusion, we provide the first evidence for digenic inheritance of a severe EBS phenotype, suggesting oligogenic inheritance as a possible explanation for the phenotypic intrafamilial variability often observed in EBS.

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Studies of a photosensitive form trichothiodystrophy case with fourth novel homozygous mutation in rare TTDA gene suggested a role of TTDA in development of atopic diathesis

M Matsuda, T Hamada, S Sakaguchi, N Ishii, M Furumura and T Hashimoto *Dermatology, Kurume University School of Medicine, and Kurume University Institute of Cutaneous Cell Biology, Fukuoka, Japan*

Trichothiodystrophy (TTD) is a rare autosomal recessive disorder, characterized by ichthyosis, sulfur-deficient brittle hair and various neuro-ectodermal symptoms. Photosensitivity is present in half of patients. The photosensitive form TTD demonstrates genetic heterogeneity with about 20 different mutations in XPD, XPB or TTDA genes, encoding distinct subunits of general transcription/repair factor IIH (TFIIH), involving in both NER and transcription. Approximately 80% cases have XPD mutations, whereas only three mutations in TTDA gene have been published. We identified a fourth novel TTDA mutation in a 13-year-old Japanese male patient, who were born as a collodion baby, and developed ichthyosiform erythroderma, accompanied by severe itching, brittle hair, short stature and photosensitivity. He had atopic diathesis with persistent eosinophilia and elevated serum IgE level. Histopathology for ichthyotic skin showed epidermal acanthosis with detaching parakeratotic stratum corneum and absence of granular layer, and dense lympho-eosinophilic infiltrate in the upper dermis. Direct nucleotide sequencing revealed a homozygous substitution (c.163G>T) at exon 2 in TTDA gene, which leads to a novel nonsense mutation p.Glu55X. The mechanism for abnormal epidermal differentiation in TTD remains unclear. Immunohistochmically, we previously demonstrated decreased expression of loriclin, and increased expression of involucrin, but normal level of filaggrin, in the skin of another TTD case with a XPD mutation. The present case showed lower filaggrin expression, in addition to the changes in expression levels of other 2 differetiation-related proteins. Because no Japanese population-specific FLG gene mutation was found in this case, we speculated that mutations in TTD-related genes may cause abnormal epidermal differentiation, resulting in development of atopic diathesis.

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Arylhydrocarbon receptor repressor (AhRR) function revisited: Repression of CYP1 activity in human skin fibroblasts is not related to AhRR expression

E Fritsche,[1,2] J Tigges,[1] H Weighardt,[1] S Wolff,[1] C Goetz,[1] I Foerster,[1] Z Kohne,[1] U Huebenthal,[1] H Merk,[2] J Abel,[1] T Haarmann-Stemmann[1] and J Krutmann[1] *1 Leibniz Institute for Environmental Medicine, Duesseldorf, Germany and 2 Dermatology and Allergology, University Clinic RWTH, Aachen, Germany*

The skin reacts to environmental noxae by inducing cytochrome P450 (CYP)-catalyzed reactions via activation of the aryl hydrocarbon receptor (AhR). A drawback of this response is generation of oxidative stress, which is especially dangerous for postreplicative cells like dermal fibroblasts, in which damage may accumulate over time. Accordingly, in dermal fibroblasts, CYP1 expression is repressed, and it has been proposed that this is due to the aryl hydrocarbon receptor repressor (AhRR), which is supposedly overexpressed in fibroblasts as compared to other skin cells. Here, we revisited this 'AhRR hypothesis', which has been mainly based on ectopic overexpression studies and correlation analyses of high AhRR gene expression with CYP1A1 repression in certain cell types. In primary human skin fibroblasts (NHDF) of 25 individuals we found that against the common view (i) the AhRR was expressed only at moderate RNA copy numbers and that (ii) in some fibroblast strains CYP1A1 mRNA expression could be induced by AhR activators. However, even highest induction did not translate into measurable CYP1 enzyme activity, and neither basal expression nor mRNA inducibility correlated with AhRR expression. Also, enhancement of CYP1A1 mRNA expression by trichostatin A, which inhibits AhRR-recruited histone deacetylases at the CYP1A1 promoter, failed to induce measurable CYP1 activity. Finally, AhRR deficient (-/-) murine embryonic fibroblasts were not induced to biologically relevant CYP1 enzyme activity despite impressive mRNA induction, because even the induced CYP1A1 copy numbers – similar to the human samples – were very low. These data clearly indicate that repressed CYP1 activity in NHDF is not causally related to AhRR expression, which may serve a different, yet unknown biological function.

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Novel and recurrent mutations in Japanese patients with focal dermal hypoplasia

G Nakanishi,[1] K Hasegawa,[2] T Oono,[3] S Koshida,[4] K Iwatsuki[5] and T Tanaka[1] *1 Dermatology, Shiga University of Medical Science, Otsu, Japan, 2 Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, 3 Nutritional Science, Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki, Japan, 4 Pediatrics, Shiga University of Medical Science, Otsu, Japan and 5 Dermatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*

Goltz syndrome or Focal dermal hypoplasia (FDH) is an X-linked dominant disorder characterized by malformations and defects affecting the skin, eyes, central nerve system, and skeleton. In 2007, mutations in PORCN gene were identified as the molecular basis of FDH and many PORCN mutations have been published so far. However, there is only one report about the FDH patient with a PORCN mutation from Japan. Here, we report the recurrent mutation, c.129G>A, which leads to a nonsense mutation, W43X, and the novel mutation, c.386delT, in two Japanese cases of FDH patients. The female patient with the recurrent mutation had typical cutaneous symptoms and skeleton abnormality, but the female patient with the novel mutation had only cutaneous symptoms with liner hypo-pigmentation along Blaschko's lines and no other systemic malformations and defects. In the latter case, DNA was isolated from peripheral blood cells, lesional skin, and non-lesional skin. The percentage of cells carrying a mutation estimated by subcloning and sequencing of the PCR product was 3.2% in peripheral blood cells, 21% in lesional skin, and 16% in non-lesional skin. RT-PCR analysis from skin samples showed PORCN mRNA of a mutated allele had 13bp nucleotides insertions created by the alternative splicing site. This mutation leads to abnormal PORCN protein with in frame insertions including eight amino acids, TTHRGTDD, instead of original four amino acids, AQMI (126-129). In our study, we identified the recurrent PORCN mutation in the typical FDH female patient and the novel mutation of somatic mosaicism in the FDH female patient of very mild phenotype.

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Germline mutation in *ATR* underlying a new autosomal dominant oropharyngeal cancer syndrome

A Tanaka,[1,2] S Weinel,[3] N Nagy,[1] M O'Driscoll,[4] JE Lai-Cheong,[1] CL Kulp-Shorten,[3] A Knable,[3] M Hide,[2] J Callen[3] and JA McGrath[1] *1 St John's Institute of Dermatology, King's College London (Guy's Campus), London, United Kingdom, 2 Department of Dermatology, Hiroshima University, Hiroshima, Japan, 3 Division of Dermatology, University of Louisville School of Medicine, Louisville, KY and 4 Genome Damage and Stability Centre, University of Sussex, Brighton, United Kingdom*

ATR (Ataxia Telangiectasia and Rad3-related) is an essential regulator of genome integrity. It controls and co-ordinates DNA replication origin firing, replication fork stability, cell cycle checkpoints and DNA repair. Previously, autosomal recessive loss-of-function mutations in *ATR* have been demonstrated in the developmental disorder Seckel syndrome. Here, however, we report a different kind of genetic disorder due to functionally compromised ATR activity, translating into an autosomal dominantly inherited disease. The condition affects 24 individuals in a 5 generation pedigree, and comprises oropharyngeal cancer, skin telangiectases and mild developmental anomalies of hair, teeth and nails. We mapped the disorder to a ~16.8 cM interval on 3q22-24 and by sequencing candidate genes identified a heterozygous missense mutation (c.6431A>G; p.Gln2144Arg) in *ATR* that segregated with the disease. The mutation occurs within the FAT (FRAP, ATM and TRRAP) domain of ATR that can activate p53. The mutation did not lead to a reduction in *ATR* gene expression but cultured fibroblasts demonstrated reduced p53 levels after activation of ATR with hydroxyurea in comparison to normal control fibroblasts. Moreover, loss-of-heterozygosity for the ATR locus was noted in oropharyngeal tumor tissue. Collectively, the clinicopathologic and molecular findings identify a new cancer syndrome and provide evidence implicating a germline mutation in *ATR* and susceptibility to malignancy in humans.

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Elucidation of the molecular basis of a novel osteocutaneous disease

O Sarig,[1] S Nahum,[2] D Rapaport,[3] A Ishida-Yamamoto,[4] D Fuchs,[1] L Qiaoli,[5] K Cohen,[2] R Spiegel,[6] J Nousbeck,[1] S Israeli,[1] Z Borochowitz,[7] G Padalon,[1] J Uitto,[5] M Horowitz,[3] S Shalev[6] and E Sprecher[1] *1 Tel Aviv Medical Center, Tel Aviv, Israel, 2 Technion, Haifa, Israel, 3 Tel Aviv University, Tel Aviv, Israel, 4 Asahikawa University, Asahikawa, Japan, 5 Jefferson Medical College, Philadelphia, PA, 6 Haemek Medical Center, Afula, Israel and 7 Bne Zion Medical Center, Haifa, Israel*

We studied on a novel autosomal recessive osteocutaneous disorder that we termed tricho-onycho-skeletal (TOS) syndrome. Clinical characterization of the syndrome in 2 families revealed a number of key features including growth restriction, facial dysmorphism, hypoplastic fingernails and sparse hair. Using homozygosity mapping, we located the disease gene to 3p21.2-3p21.31. We then performed a whole exome sequencing analysis complemented with Sanger direct sequencing of poorly covered regions. After having excluded common variations as well as having screened a total of 300 population-matched controls for each potentially pathogenic sequence alteration, we identified a homozygous point mutation (p.L171P) in POC1A (Centriolar Protein Homolog A). The mutation, which was found to co-segregate with the disease phenotype in the two families, affects a highly conserved amino acid residue and is predicted to interfere with protein function by PolyPhen-2, SIFT and align-GVGD softwares. As Poc1 was previously found to have a role in centriole stability in unicellular organisms, we used confocal microscopy and electron microscopy to ascertain centriole composition. Although centriole structure seemed to be preserved, their number and distribution was abnormal in patient fibroblasts. Corroborating this observation, genes and pathways associated with cell cycle and/or centrosome formation were found to be massively over-represented in a global gene expression study of patient cells. Accordingly, patient cells exhibited significantly slower cell-cycle progression by FACS analysis as well as abnormal trafficking of microtubule-dependent cholera toxin to the Golgi. Collectively, our data underscore the importance of POC1A for proper bone, hair and nail formation as well as normal centrosome function.

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Sirtuins and Nampt in human skin cells

Y Chen,[1] S Carpenter,[2] G Heenan,[1] J Lyga[1] and R Wyborski[1] *1 Avon Products., Suffern, NY and 2 Elusys Therapeutics, Inc., Pine Brook, NJ*

Sirtuins are NAD-dependent histone deacetylases that are involved in various cellular processes, including DNA repair, genome stability, energy production and metabolism, stress resistance and cell survival. Sirtuin activity is NAD-dependent and Nicotinamide phosphoribosyltransferase (Nampt) is the rate limiting enzyme in NAD biogenesis. Previously we have demonstrated that Sirtuin1 and Sirtuin3 gene expressions are significantly affected by both intrinsic and extrinsic aging factors. In this study, we were interested in evaluating the effect of aging, UV exposure, and oxidative stress on the expression of other Sirtuin family genes. We also investigated the role of Nampt in regulating Sirtuin functions by siRNA knockdown studies. Human epidermal keratinocytes and human dermal fibroblasts from different age donors were obtained and maintained in culture. UV treatment was carried out with single wavelength UVB or UVA lamps and irradiation intensity was measured using radiometer with corresponding filters. Oxidative stress was achieved through H_2O_2 treatment. Gene expression analysis was performed using Taqman primer and probes. We discovered that with aging, there is a dramatic decrease in Sirtuins and Nampt gene expressions. UV exposure and oxidative stress also significantly alter Sirtuins and Nampt gene expression, but the response is gene specific. Furthermore, siRNA knockdown of Nampt in the fibroblast lead to down-regulation of both Sirtuin1 and Sirtuin3 genes. It also significantly changed the NAD/NADH ratio in the mitochondria. During the course of skin aging, both intrinsic aging and photoaging play important roles. Sirtuin proteins play important roles in cell cycle regulation, metabolism, genome stability, and stress resistance. Nampt is crucial in maintaining Sirtuin1 and Sirtuin3 level, and is very important in mitochondria activity and efficiency. We postulate that by stimulating Sirtuins and Nampt gene expression, it may be possible to modulate skin aging by increasing the cells' ability to response better to both intrinsic and extrinsic stress.

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HDAC inhibition prevents loss of subcutaneous fat in CSB-deficient mice

M Majora, C Goetz, C Schumacher, M Schneider and J Krutmann *Molecular Aging Research, IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany*

Cockayne syndrome (CS) is a progeroid disease caused by mutations in either the CSB or CSA gene. Cells lacking a functional CSB protein are characterized by compromised DNA repair and inability to mount normal transcriptional responses towards UV radiation. We have previously shown that CSB-deficient hairless mice are resistant against wrinkle formation upon chronic UVA irradiation but similar to CS patients are prone to loss of subcutaneous fat. These findings correlated with decreased expression of the major murine collagenase MMP-13 and increased expression of the anti-adipogenic enzyme MMP-3 in the skin of UVA-irradiated CSB-deficient mice in comparison to wildtype mice. According to that, a single dose UVA irradiation of CSB-deficient and wildtype MEFs resulted in a similar phenotype with regard to the differential expression of MMP-13 and MMP-3. We now report that the UVA-induced aberrant gene expression profile in CSB-deficient MEFs was accompanied by an increased expression and phosphorylation of c-Jun, a factor which is known to be involved in transcriptional regulation of various MMP genes. Interestingly, treatment with the histone deacetylase (HDAC) inhibitor SAHA could restore the abnormal expression levels of MMP-13 and MMP-3 in CSB-deficient cells to wildtype levels upon UVA irradiation. While low levels of SAHA tend to stimulate expression of MMP-13 and MMP-3 in both CSB-deficient and wildtype MEFs high doses inhibit induction of both genes. In parallel, low levels of SAHA enhance expression and phosphorylation of c-Jun while high doses block c-Jun accumulation. Even more important, oral administration of SAHA significantly reduced spontaneous as well as UVA radiation-induced loss of subcutaneous fat *in vivo* in CSB-deficient mice. These data indicate that the defective transcriptional response towards UVA radiation in CSB-deficient cells can be normalized by HDAC inhibitors at least for some genes. Moreover, they suggest that aberrant protein acetylation may be a novel molecular mechanism contributing to the pathology of CS.

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Evidence for interplay between hypoxia and epigenetic dysregulation in the pathogenesis of cerebellar neurodegeneration in CSB deficient mice

C Schumacher, M Majora, T Schreiber, E Fritsche and J Krutmann *Molecular Aging Research, IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany*

Cockayne syndrome (CS) is a progeroid disease characterized by progressive multi organ dysfunction with degeneration of the CNS especially pronounced in the cerebellum. CS is caused by mutations in the CSA or CSB gene. Cells lacking a functional CSB protein are compromised in repairing certain forms of DNA lesions including those induced by UV radiation in actively transcribed genes. Although often regarded as DNA repair deficiency syndrome, several symptoms of CS including cerebellar degeneration are hardly explainable by unrepaired DNA damage therefore suggesting additional functions of the CSB protein. In this regard, we and others have recently provided evidence that CSB-/- skin cells fail to mount a gene expression response induced by hypoxic stress. As the cerebellum is exquisitely sensitive towards lack of oxygen we hypothesized that cerebellar degeneration in CS could be due to inability to mount an appropriate hypoxic response. Using CSB-/- mice as a model, we found that these animals when compared to wildtype mice showed less Purkinje cells, a decreased expression of the hypoxia-inducible gene VEGF and a reduced number of blood vessels. Moreover, reduced expression of the hypoxia-inducible histone acetyltransferase and HIF-1 cofactor p300 was detected in CSB-/- Purkinje cells. In addition, an impaired transcriptional response including expression of VEGF, GLUT-1 and HMOX1 towards hypoxia was also noted in neurospheres prepared from the cerebellum, but not the cerebrum of fetal CSB-/- mice. Interestingly, the compromised hypoxic response in CSB-/- cerebellar neurospheres could be rescued if cultures were treated with the histone deacetylase inhibitor SAHA. These studies provide *in vivo* and *in vitro* evidence for a defective hypoxic response in the cerebellum of CSB-/- mice. They also support the concept that the CSB protein, in addition to its role in DNA repair, serves an important epigenetic regulatory function and thereby controls gene expression.

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Discovery of regulatory-element risk variants in an ethnic-specific atopic dermatitis cohort using pooled targeted deep sequencing

KJ Gulewicz,[1] D Albea,[1] I Oh,[1] A Bowcock,[2] A Shemer,[4] JG Krueger,[5] E Guttman-Yassky[3] and C de Guzman Strong[1] *1 Medicine/Dermatology/Pharmacogenomics, Washington University School of Medicine, St. Louis, MO, 2 Genetics, Washington University School of Medicine, St. Louis, MO, 3 Dermatology, Mt. Sinai School of Medicine, New York, NY, 4 Tel-Aviv University, Tel-Aviv, Israel and 5 The Rockefeller University, New York, NY*

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with pruritus, scaling, and erythema. Filaggrin (FLG) mutations account for up to 50% of moderate to severe AD in Europeans but little is known about the genetic risk factors in other ethnic minorities that are disproportionately affected by AD. Moreover, exclusion of common FLG mutations and persistent strong association to the Epidermal Differentiation Complex (EDC) suggest additional risk variants for the remaining 50% heritability. We previously described a case cohort of Middle Eastern descent with the moderate to severe AD phenotype that is characterized by broad epidermal terminal differentiation defects and is negative for the common FLG mutations. We hypothesize that risk variants in this cohort reside in regulatory regions that are in linkage disequilibrium (LD) with FLG or GWAS-tagging SNPs and affect global EDC expression. Using Haploview and ENCODE data, we identified 10 regulatory regions in LD with either rs877776 (AD-GWAS-tagging SNP near FLG/HRNR) or rs7927894 (AD-GWAS-tagging SNP on 11q13). Targeted deep sequencing of these regulatory elements in pooled patient DNA samples identified differential SNPs compared to reference genomes. We are currently investigating the significance of these SNPs as well as the contribution of other FLG mutations or copy number variations in this cohort. In sum, the approach of prioritizing these regulatory elements for targeted deep sequencing represents a novel and potentially high yield source of AD associated variants. This will contribute to a better understanding of population-specific genetic regulation of AD within ethnic minorities and allow for the development of more effective and personalized treatments.

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A photoaging-like phenotype in dermal equivalents: Evidence for proteasomal dysregulation in KSS fibroblasts

C Goetz,[1] B Schuermann,[1] M Majora,[1] S Franke,[1] M Schneider,[1] F Bernerd[2] and J Krutmann[1] *1 Molecular Aging Research, IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany and 2 L'Oreal Recherche, Clichy, France*

As mutations of mitochondrial (mt) DNA are frequently detected in aged tissues it has been suggested that they are causally related to the aging process but mechanistic details still remain largely unknown. We have previously shown that human dermal equivalents (DEs) containing fibroblasts from Kearns-Sayre syndrome (KSS) patients harboring high levels of UV-inducible large scale deletions of the mtDNA develop a phenotype resembling photoaged human skin. Compared to DEs containing normal human fibroblasts (NHF), KSS DEs show elevated levels of reactive oxygen species (ROS), increased expression of the collagenase MMP-1 and progressive loss of intact collagen fibers. Moreover, increased expression of VEGF, lysyl oxidase (LOX) and IL-8 was also noted in KSS DEs. We now provide evidence that the photoaging-like phenotype in KSS DEs is caused by proteasomal dysfunction and postulate a functional link between mtDNA deletions, elevated ROS levels, proteasomal activity and aberrant gene expression. To verify our hypothesis, KSS and NHF DEs were treated with sulforaphane (SF), a substance which is known to increase proteasomal activity. SF treatment not only resulted in reduction of the elevated mtROS levels in KSS fibroblasts but also induced a significant reduction of MMP-1 expression in KSS DEs which was not seen in NHF DEs. Moreover, SF also significantly reduced expression of VEGF, LOX and IL-8 in KSS DEs but had no suppressive effect on the expression of these genes in NHF DEs. In contrast, exposure to the proteasome inhibitor bortezomib (BZ) resulted in substantially elevated mtROS levels and strongly increased expression of MMP-1 in NHF DEs while KSS DEs were hardly affected by BZ. Taken together our data confirm recent studies proposing a functional interplay between mitochondria and the proteasome. Moreover, they suggest a causal relationship between mtDNA mutagenesis, ROS, proteasomal dysfunction, altered gene transcription and a photoaging-like phenotype.

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Restoration of XPC protein and induction of DNA repair in homozygous and heterozygous xeroderma pigmentosum group C cells by readthrough of stop codons using aminoglycoside compounds

C Kuschal, JJ DiGiovanna, SG Khan and KH Kraemer *Derm Branch, NCI, Bethesda, MD*

Xeroderma pigmentosum (XP) is a rare recessive disorder with sun sensitivity and a 10,000-fold increase in skin cancers due to defective nucleotide excision repair. Many XP patients have premature termination codon (PTC) mutations in the XPC gene. In-vitro studies demonstrated that aminoglycosides like Geneticin are able to "read through" PTC mutations, leading to partial induction of missing proteins. We recently showed that levels of Geneticin that reduced cell survival by 50% can partially restore XPC protein expression and induce DNA repair in 2 XPC-deficient cell lines with homozygous CGA Arg155>X or CGA Arg 220>X PTC mutations. We now report on the efficiency of Geneticin in 4 compound heterozygote XPC cell lines containing only 1 PTC mutation. All cells tested have no XPC protein and no detectable repair of UV-induced 6-4 photoproducts (6-4PP) or cyclobutane pyrimidine dimers (CPD). In Geneticin treated cells containing CGA Arg155>X, CGA Arg 415>X, and AAG Lys 692>X but not in cells with AAA Lys522>X, XPC protein, recruitment of XPB and XPD proteins and removal of 6-4PP was visualized at sites of UV damage. However XP protein recruitment and 6-4PP removal was less efficient than in the Arg >X homozygous cells. Unlike the Arg>X homozygous cells, Geneticin had no effect on the repair of CPD in the compound heterozygotes. In the Arg>X homozygous cells we detected increased readthrough with the levels of aminoglycoside antibiotic, Gentamycin that reduced cell survival by only 20-30%, but the effect was lower than with Geneticin. Aminoglycoside compounds can induce readthrough of homozygous and heterozygous PTC mutations in XPC cells however, the efficiency was dependent on the specific PTC mutation and type of compound. Since topical Gentamycin is in general use, further studies may be warranted to determine if Gentamycin can be used to increase DNA repair in skin of patients with XPC PTC mutations.

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Patients with the rare DNA repair disease overlap syndrome xeroderma pigmentosum and trichothiodystrophy (XP/TTD) are at high risk for skin and internal cancers

SG Khan,[1] D Tamura,[1] T Rao,[1] WM Zein,[2] BP Brooks,[2] J Boyle,[1] T Ueda,[1] JJ DiGiovanna[1] and KH Kraemer[1] *1 Derm Branch, NCI, Bethesda, MD and 2 Ophthal Gen Br, NEI, Bethesda, MD*

Mutations in the XPD DNA repair gene are associated with rare autosomal recessive genetic diseases: trichothiodystrophy (TTD) and xeroderma pigmentosum (XP). While TTD patients have a wide spectrum of clinical manifestations and developmental abnormalities without skin cancer, XP patients have 10,000-fold increased skin cancer risk. We describe 5 patients from 3 different families who are compound heterozygotes for mutations in XPD and have features of both TTD and XP including skin and internal cancers. All these patients had acute burning on minimal sun exposure, freckle-like pigmentation on sun-exposed skin, cataracts and tiger-tail banding of the hair shafts under polarized light. Three sisters in family 1 had skin cancer: 35 y/o XPTTD64BE had 3 BCCs and 1 SCCs since age 20; 24 y/o XPTTD385BE developed 1 BCC at age 24; and XPTTD384BE died of metastatic melanoma with unknown primary at age 32. XPTTD464BE in family 2 is an 18 y/o female with mild cognitive delays, who had a large anaplastic hemangiopericytoma of the brain at age 17. XPTTD465BE in family 3 is a 55 y/o male who began developing skin cancers at age 20 yr. He had a total of 25 skin cancers including 5 melanomas with metastasis to his parotid glands. Families 1 and 2 had the same common functionally null p.R616P mutation which was reported in both XP and TTD. The second allele in family 1 was p.R601W, while family 2 had p.R658G. XPTTD465BE had previously reported XP mutation p.R683C from one allele and a premature stop codon (Q662X) from the other allele. These results suggest that XP/TTD patients are at increased risk for skin and internal cancers and these cancers may be very aggressive. Each XPD mutation might affect different functions of TFIIH and thus the combination of mutations may be critical for distinct clinical features in these patients.

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Expanding the differential diagnosis for pachyonychia congenita

NJ Wilson,[1] CD Hansen,[2] D Azkur,[3] CN Kocabas,[3] A Metin,[3] Z Coskun,[3] ME Schwartz,[4] PR Hull,[5] WH McLean[1] and FJ Smith[1] *1 Molecular Medicine, University of Dundee, Dundee, United Kingdom, 2 Dermatology, University Utah, Salt Lake City, UT, 3 Ankara Children's Health and Hematology Oncology Hospital, Ankara, Turkey, 4 PC Project, Salt Lake City, UT and 5 Dermatology, University of Saskatchewan, Saskatoon, SK, Canada*

Nail dystrophy is a hallmark of the autosomal dominant disorder pachyonychia congenita (PC), accompanied by keratoderma and other ectodermal defects. We recently analyzed several cases initially diagnosed as PC but who presented with nail dysplasia without other ectodermal abnormalities. None had mutations in any of the 5 keratin genes associated with PC (KRT6A, KRT6B, KRT6C, KRT16 & KRT17) or the gene encoding connexin-30 (Clouston syndrome is part of the differential diagnosis for PC). The frizzled 6 gene (FZD6) was analyzed because mutations in this gene were recently shown to cause autosomal recessive nail dysplasia. FZD6 mutations were identified in 3 unrelated families with clinically similar nail dystrophy, where all fingernails and toenails were discolored and thickened from birth. An affected boy born to healthy consanguineous parents was homozygous for mutation p.Arg509X; whereas unaffected parents and sibling were heterozygous carriers. In a second family, the affected individual was also homozygous for p.Arg509X. In the third family, the proband was compound heterozygous for p.Arg96Cys/p.Glu438Lys. Wnt signaling is important for the development of ectodermal appendages including nails. FZD6 belongs to a family of receptors in the Wnt pathway and is expressed in the ventral nail matrix and nail bed. Mutations in the FZD agonist R-spondin4, also in the Wnt pathway, cause autosomal recessive anonychia. These results highlight the difficulty in diagnosing rare disorders where clinical features overlap. We conclude that FZD6 should be added to the screening panel for PC and should especially be considered in sporadic or confirmed recessive cases of isolated nail dysplasia where all nails are discolored and thickened from birth. Further Wnt signaling molecules are possible candidate genes for hereditary nail dystrophies.

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The pseudoxanthoma elasticum phenotype in the *Abcc6*$^{-/-}$ mice is modified by different genetic backgrounds

Q Li, A Donahue and J Uitto *Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA*

Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder characterized by ectopic mineralization of connective tissues in skin, eyes and cardiovascular system. This disease is caused by mutations in the *ABCC6* gene. *Abcc6*$^{-/-}$ mice on mixed 129S1/SvImJ and C57BL/6J backgrounds were initially generated by targeted ablation of the mouse *Abcc6* gene, and these mice develop progressive mineralization mimicking human PXE. To investigate the hypothesis that genetic background affects the mineralization phenotype in this mouse model for PXE, the initial *Abcc6*$^{-/-}$ mice were backcrossed for five generations into C57BL/6J background (N5). The onset and degree of mineralization of connective capsule of vibrissae, a biomarker of the mineralization process in PXE, was examined in histological sections of muzzle skin containing vibrissae. The mineralization of the vibrissae in the initial *Abcc6*$^{-/-}$ mice takes place at ~6 weeks of age and is progressive. However, the onset of mineralization in *Abcc6*$^{-/-}$ mice on C57BL/6J background (N5) was delayed until between 3-4 months of age, suggesting that the genetic background plays a role in modifying the mineralization process. In addition, *Abcc6*$^{-/-}$ mice on C57BL/6J background (N5) were crossed with 129S1/SvImJ mouse strain and then interbred to generate *Abcc6*$^{-/-}$ mice. These *Abcc6*$^{-/-}$ mice developed mineralization in the vibrissae as early as 6-9 weeks and mineralization is significantly enhanced at 2 months of age. These findings suggest that modifier loci in the 129S1/SvImJ strain can modify the mineralization phenotype in the *Abcc6*$^{-/-}$ mouse model for PXE.

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Aged fibroblast gene array-looking for new affected transcripts

T Muthny, R Slavkovsky and V Velebny *Contipro Biotech s.r.o., Dolni Dobrouc, Czech Republic*

Both in dermatology and cosmetology, new strategies are constantly prospected. Thus skin genetics research is emphasized and gene expression changes related to skin aging can provide useful information. In this study, we therefore compared gene expression profiles of normal human dermal fibroblasts of young (NHDF-Y, 7±1 years old; n=4) and aged (NHDF-A, 62±2 years old; n=6) donors. Skin samples (eyelids and auricles) were obtained during plastic surgery. Fibroblasts were isolated and cultivated in a standard medium containing bovine serum (10%) and viability was determined. Total RNA was isolated, amplified, labeled, and hybridized to whole genome microarray chip. DAVID NIAID/NIH (Functional Annotation Tool) was used for identification and classification of impacted structures and biological processes. One sample t-test was performed for statistical analysis. NHDF-A proliferation rate was half compared to NHDF-Y. Performing microarray assay about 19 000 genes was detected. Setting the statistical cut off on gene expression in NHDF-A higher than 200% and lower than 50% compared to NHDF-Y, about 350 activated and 250 inhibited genes was revealed. Among up-regulated genes, functional groups as signal, glycoprotein or disulfide bond were the most affected. Extracellular matrix, collagen or basement membrane were the most common functional groups from down-regulated genes. Moreover, some specific genes (not well described in aging processes) were affected. These include lon peptidase 1 (35%-58%, mitochondrial metabolism), collagen- and calcium-binding EGF domains 1 (152%-302%, extracellular matrix remodeling), proprotein convertase subtilisin/kexin type 5 (156%-407%, integrin posttranslational modifications) etc. ($p<0.05$). In conclusion, some of significantly changed transcripts represent very interesting and novel results in term of searching of new targets both for medical and cosmetic care.

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Samd9L, a mouse paralog of _Samd9_, does not modify the mineralization process in _Abcc6_$^{-/-}$ mice, a model for pseudoxanthoma elasticum

Q Li,[1] H Guo,[1] H Matsui,[2] H Honda,[2] T Inaba,[2] E Sprecher[3] and J Uitto[1] *1 Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA, 2 Hiroshima University, Hiroshima, Japan and 3 Tel Aviv Sourasky Medical Center, Tel Aviv, Israel*

Familial tumoral calcinosis (FTC) is a group of heritable disorders characterized by ectopic mineralization of the extracellular matrix of connective tissues. The normophosphatemic variant, NFTC, is caused by mutations in the sterile α motif domain-containing 9 gene *(SAMD9)* which encodes a cytoplasmic protein with currently unknown function. This gene is located in the human chromosome 7q21, and next to it in head-to-tail orientation is a paralogous gene, *SAMD9*-like *(SAMD9)*. An interesting feature of this gene/protein system is that the *Samd9* gene is absent in mouse genome due to genomic rearrangement during evolution, and it has been suggested that *Samd9L* substitutes in mouse for Samd9 as a mineralization associated gene. In this study, we have crossed *Abcc6*$^{-/-}$ knockout mice, which demonstrate characteristic mineralization of connective tissues, with mice homozygous, heterozygous or wild-type for *Samd9L* in order to examine the contribution of *Samd9L* to the mineralization/anti-mineralization network. Examination of *Samd9L*$^{-/-}$ mice up to 8 months of age did not reveal any evidence of soft tissue mineralization. Furthermore, examination of the mineralization of the connective tissue sheath of vibrissae in *Abcc6*$^{-/-}$/*Samd9L*$^{-/-}$ mice by histopathologic stains or by direct chemical assay of calcium and phosphate showed similar degree of mineralization as in *Abcc6*$^{-/-}$ mice alone. Thus, the mouse paralog *Samd9L* of human *SAMD9* does not provide the functional redundancy required to prevent ectopic mineralization in wild-type mice under normal calcium phosphate homeostatic conditions.

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MotifMap: Integrative genome-wide maps of regulatory motif sites for model species

K Daily,[1,2,3] VR Patel,[2,3] P Rigor,[2,3] X Xie[2,3] and P Baldi[2,3,4] *1 Dermatology Branch, NCI, National Institutes of Health, Bethesda, MD, 2 Department of Computer Science, UC Irvine, Irvine, CA, 3 Institute for Genomics and Bioinformatics, UC Irvine, Irvine, CA and 4 Department of Developmental and Cell Biology, UC Irvine, Irvine, CA*

A central challenge of biology is to map and understand gene regulation on a genome-wide scale. For any given genome, only a small fraction of the regulatory elements embedded in the DNA sequence have been characterized, and there is great interest in developing computational methods to systematically map all these elements and understand their relationships. Such computational efforts are significantly hindered by the overwhelming size of non-coding regions and the statistical variability and complex spatial organizations of regulatory elements and interactions. Genome-wide catalogs of regulatory elements for all model species simply do not yet exist. The MotifMap system uses databases including 2,000 transcription factor binding motifs, refined genome alignments, and a comparative genomic statistical approach to provide novel comprehensive maps of candidate regulatory elements encoded in the genomes of model species including yeast, fly, worm, mouse, and human. The total number of predicted regulatory elements across the genomes ranges from hundreds of thousands for the yeast, worm, and fly genomes to millions for the mouse and human genomes. We assess the maps in several ways, for instance using high-throughput experimental ChIP-seq data and AUC statistics, providing strong evidence for their accuracy and coverage. MotifMap and its integration with other "omics" data provide a foundation for analyzing regulation on a genome-wide scale and automatically generating regulatory pathways and hypotheses. We demonstrate the power of this approach using the P53 apoptotic pathway and the Gli hedgehog pathway as examples. Furthermore, the binding site locations for a number of additional transcription factors implicated in skin diseases, for example Ovol2, Grhl3, STAT1, Myc, Nrf2, and Atf2, are available for multiple species.

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Cutaneous features of pseudoxanthoma elasticum in generalized arterial calcification of infancy due to _ENPP1_ gene mutations

Q Li,[1] W Schumacher,[2] D Siegel[2] and J Uitto[1] *1 Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA and 2 Dermatology, Medical College of Wisconsin, Milwaukee, WI*

Pseudoxanthoma elasticum (PXE), an autosomal recessive disorder, manifests with cutaneous lesions consisting of yellowish papules coalescing into plaques of inelastic skin. Histopathology demonstrates accumulation of pleiomorphic elastic structures with progressive mineralization. The cutaneous features are associated with characteristic ocular findings and vascular mineralization with considerable morbidity and mortality. PXE is caused by mutations in the *ABCC6* gene, which encodes an efflux transporter primarily expressed in the liver. Generalized arterial calcification of infancy (GACI) manifests at birth with extensive mineralization of large and medium sized arteries. Most infants die from myocardial infarction within the first year of life. GACI is caused by mutations in the *ENPP1* gene which encodes ecto-nucleotide pyrophosphatase/phosphodiesterase1 enzyme converting ATP to AMP and pyrophosphate. The Keutel syndrome is an autosomal recessive disease characterized by diffuse cartilage calcification, associated with developmental disorders and caused by mutations in the gene encoding matrix gla protein (MGP). We examined a 2-year old patient with PXE-like cutaneous features in the neck, inguinal folds and lower abdomen, with characteristic histopathology. The patient also had extensive vascular mineralization. The cause of tissue mineralization was evaluated by sequencing *ABCC6*, *ENPP1* and *MGP*. No pathogenetic mutations were found in *ABCC6* or *MGP*, while sequencing of *ENPP1* disclosed a homozygous missense mutation, p.Y513C, associated with GACI. Thus, this study demonstrates the presence of cutaneous features of PXE in a genetically distinct disease, GACI, and expands the spectrum of PXE-related disorders. Our findings also attest to the complexity of mineralization/anti-mineralization networks in skin, with mutations in different genes resulting in similar phenotypic presentations.

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Intravenously injected recombinant human type VII collagen restores collagen function in dystrophic epidermolysis bullosa

X Wang,[1] M Amir,[1] B Hwang,[1] D Keene,[2] Y Hou,[1] A Bauskar,[1] D Woodley[1] and M Chen[1] *1 Dermatology, University of Southern California, Los Angeles, CA and 2 Shriners Hospital for Children, Portland, OR*

Patients with dystrophic epidermolysis bullosa (DEB) have incurable skin fragility, blistering and multiple skin wounds because of mutations in the gene that encodes for type VII collagen (C7). We showed previously that intravenously (IV) injected, molecularly-engineered DEB fibroblasts (overexpressing human C7) homed to the animal's wound and continuously delivered C7 to the basement membrane zone (BMZ) of the host's skin at the wound site. In this study, we evaluated the feasibility of IV injection of recombinant C7 protein for DEB treatment. We first made a wound on the back of athymic nude mice and then IV injected these mice with recombinant human C7. Surprisingly, the injected C7 trafficked to the wounded skin and incorporated into the newly formed BMZ of the mouse's skin. In contrast, we did not detect human C7 expression in unwounded skin areas or other organs. Most interestingly, the wounds of mice receiving IV C7 demonstrated accelerated wound healing compared with BSA injected control mice. To evaluate the feasibility of this approach in the disease models, we grafted immuno-deficient mice with either RDEB skin tissues regenerated from RDEB keratinocytes and accelluar dermis or murine DEB-like skin from knock out C7 mice and then IV injected the mice with recombinant C7. We found that the IV injected human C7 specifically homed to the engrafted DEB skin and incorporated into its BMZ. Unlike the skin of normal mice, the engrafted DEB-like skin did not require prior wounding for the IV-injected C7 to home to it, incorporate into its BMZ, and correct the RDEB clinical phenotype. Our studies provide the first evidence for using an IV-based protein therapy approach to correct a skin disease in a preclinical animal model. In addition to healing chronic wounds, this strategy may be particularly useful for patients with DEB who have multiple open wounds and skin fragility.

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AP-1 proteins are induced by irritant and field injury through purinoceptor and EGFR activation

KJ White,[1] VJ Maffei[1] and RA Swerlick[1,2] *1 Dermatology, Emory University, Atlanta, GA and 2 Dermatology, Atlanta Veterans Affairs Hospital, Decatur, GA*

Early molecular events mediating irritant skin injury (ISI) remain largely undefined. We previously established that the model irritant sodium lauryl sulfate (SLS) causes ATP release, purinoceptor activation, and metalloprotease-dependent activation of the epidermal growth factor receptor (EGFR) in keratinocytes leading to pro-inflammatory gene induction. Further research using SLS and purine nucleotides (NTD) in HaCaT keratinocytes suggests that activator protein (AP)-1 transcription factors may serve as molecular markers for irritant and field injury. AP-1 proteins are involved in induction of genes responsible for angiogenesis, hyperproliferation, and inflammation and have been clearly linked to inflammatory skin diseases. Gene array analysis of SLS-treated skin and HaCaT cells demonstrated rapid and robust induction of AP-1 mRNAs including cFos, cJun, and ATF3. Furthermore, both SLS and purine NTDs induce AP-1 proteins *de novo in vitro*, and streptavidin-agarose pull-down assays suggest the proteins are activated and capable of binding oligonucleotides relevant for gene regulation. Further study into the mechanism behind irritant-mediated AP-1 induction reveals that brefeldin A inhibits SLS-induced cFos protein, indicative of a role for ATP release. Involvement of NTDs and purinoceptors was further supported by prevention of SLS and ATP induction of cFos by apyrase pretreatment *in vitro*. In addition, pretreatment with marimastat, a broad spectrum metalloprotease inhibitor, or cetuximab, a specific EGFR monoclonal antibody, prevented SLS- and purine NTD-induced cFos expression. Anti-EGFR treatment had no effect on ATF3 induction suggesting concurrent activation of an EGFR-independent pathway by SLS and purine NTDs. In summary, irritant injury to skin induces AP-1 protein expression *in vivo* and *in vitro* through EGFR-dependent and -independent mechanisms. Our data provide novel evidence for involvement of NTDs in AP-1 induction by SLS in keratinocytes and insight into the early mechanisms of ISI.

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Re-expression of murine _Abcc6_ in the liver counteracts ectopic mineralization in a mouse model of pseudoxanthoma elasticum _(Abcc6$^{-/-}$)_

Q Jiang, S Takahagi, D Wang and J Uitto *Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA*

Pseudoxanthoma elasticum (PXE) is an autosomal recessive multi-system disorder characterized by progressive ectopic connective tissue mineralization, caused by mutations in the *ABCC6* gene which is expressed primarily in the liver. Although the pathomechanisms resulting in ectopic mineralization remain unclear, one would expect that reconstitution of functional ABCC6 activity in the liver may counteract ectopic mineralization in PXE. To test this hypothesis, we delivered an expression construct containing liver-specific promoter (albumin) and full-length mouse *Abcc6* cDNA to the liver of immunodeficient *Abcc6*$^{-/-}$ mice generated by crossing them with *Rag*^{1} mice. In this model, mineralization of the connective tissue sheath surrounding the vibrissae is noted as early as 5-6 weeks of age, a biomarker of the generalized mineralization process. Bi-weekly administration of *mAbcc6* expression vector was performed to the mice initially at the age of 4 weeks to study prevention or at the age of 12 weeks to examine reversal of the mineralization through hydrodynamic tail vein injection, for a total of 8 or 10 weeks, respectively. Meanwhile, parallel control groups were administered with a LacZ expression construct in the same targeting vector, and liver specific targeting of the construct was confirmed by beta-galactosidase staining. Progressive mineralization of vibrissae, a characteristic feature of *Abcc6*$^{-/-}$, was abrogated in the mAbcc6 treated mice both in prevention and reversal study. However, the preformed mineral deposits were not reduced in the mice in the reversal studies. The results suggest that the process of mineralization in this PXE mouse model is preventable, but not reversible, by administration of functional Abcc6 to the liver. Thus, re-expression of ABCC6 through gene therapy approach may offer a novel strategy to treat patients with PXE.

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Coactivator MED1 regulates epidermal and hair follicular keratinocyte proliferation and differentiation

L Hu,[1,2] V Bul,[1,2] DD Bikle[1,2] and Y Oda[1,2] *1 Endocrine Research, University of California San Francisco, San Francisco, CA and 2 VA Medical Center, San Francisco, CA*

The transcriptional coactivator complex Mediator facilitates transcription of nuclear hormone receptors and other transcription factors. Previously, we isolated the Mediator complex from primary keratinocytes as VDR binding proteins. The role of Mediator was examined in cultured keratinocytes. Silencing of Mediator subunits resulted in hyper-proliferation of keratinocytes and defects in keratinocyte differentiation. We then examined *in vivo* role of Mediator by generating conditional null mice, where a critical subunit of Mediator, MED1, is deleted from their keratinocytes by using a keratin 14 driven Cre-lox system. The MED1 ablation resulted in aberrant hair differentiation and cycling leading to hair loss. During the first hair cycle, MED1 deletion resulted in a rapid regression of the hair follicles. Hair differentiation was reduced, and VDR/β-catenin regulated hair differentiation genes were markedly decreased. In the subsequent adult hair cycle, MED1 ablation induced progression of hair follicle. Shh signaling components, Shh, Ptch1 and Gli, increased. However, hair keratins and BMP components such as BMP2 and 4 were lower, indicating terminal differentiation was not sufficient. Deletion of MED1 also caused hyper-proliferation of interfollicular epidermal keratinocytes, and increased the expression of epidermal differentiation markers. These results indicate that MED1 has a critical role in regulating hair and epidermal proliferation and differentiation.

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An epidermal-specific cis-regulatory enhancer in the EDC with spatial and temporal sensitivity

I Oh, D Albea, B Baker, G Kroner and C de Guzman Strong *Department of Medicine/Dermatology/Pharmacogenomics, Washington University School of Medicine, St. Louis, MO*

The Epidermal Differentiation Complex (EDC) comprises a conserved cluster of 4 gene families important for the epidermal barrier. The mechanism underlying the coordinate expression of the EDC during epidermal development is largely unknown. We previously reported a conserved non-coding element (CNE), 923, approximately 923kb downstream from the 5′ end of the EDC, that demonstrated the highest enhancer activity in our CNE screen and corroborated with high DNaseI hypersensitivity and epidermal-specific activity in transgenic reporter mice. We hypothesize a role for 923 as a locus control region or master genetic switch to activate EDC expression. An independent set of transgenic reporter mice revealed 923 enhancer activity correlating with epidermal differentiation and the patterning of barrier acquisition thus demonstrating the sensitivity of 923 to spatial and temporal cues supportive of our hypothesis. A bioinformatics screen to determine the molecular mechanism affecting 923 activity revealed identification of 4 PhastCons blocks (sequences) within 923 that are highly conserved across 28 vertebrate species and are marked by ENCODE-annotated DNaseI hypersensitivity and H3K4me1 histone modification that tags enhancers. Using reporter assays in proliferating and differentiating keratinocytes, we found a direction-dependent requirement for PhastCons block 1 and a direction-independent requirement for PhastCons block 4 for enhancer activity. CREB and AP-1 transcription factor binding sites were the top hits in PhastCons blocks 1 and 4, respectively, and have been implicated in regulating epidermal differentiation thus suggesting a role for these transcription factors in regulating 923 activity. Functional and chromosomal conformation capture assays are ongoing in support of 923's role to regulate the EDC. In sum, elucidation of the molecular mechanism activating EDC expression will enable us to better understand the pathophysiology of inflammatory skin diseases that are genetically linked to the EDC.

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Genetic and environmental factors influence the severity and tissue susceptibility of mineralization in PXE mouse models

Q Jiang, D Wang and J Uitto *Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA*

Pseudoxanthoma elasticum (PXE) is caused by mutations in the *ABCC6* gene. The clinical features of PXE and their presentation in the skin, eyes, cardiovascular, and gastrointestinal systems emphasize the fact that PXE is a systemic disease affecting many different organs in the body. However, there is considerable, both intra- and interfamilial variability in the clinical features and severity of symptoms. A question has been raised whether there are genetic and environmental modifiers working in combination with the mutant ABCC6 to explain the variability of PXE manifestations. To address this question, we studied *Abcc6*$^{-/-}$ mouse models in different genetic backgrounds or on different diets. This study revealed that deficient diet in magnesium enhances mineralization in the murine PXE model in a time-dependent manner. In addition, the *Abcc6*$^{-/-}$ mouse on mixed genetic background of C57BL/6 and 129S1/SvJ developed earlier-onset mineralization of muzzle skin (at 5 weeks of age) than the mice carrying the same mutation but on pure C57BL/6 background (at 8 weeks of age). Moreover, the *Abcc6*$^{-/-}$ mouse either on C57BL/6 or mixed C57BL/6 and 129S1/SvJ background demonstrates generalized connective tissue calcification while the PXE mouse on C3H/He genetic background shows predominately cardiac calcification. This study emphasized the complexity of PXE phenotypes at the genome/environment interface.

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mtDNA mutations seen in UV-induced mouse models of skin cancer induce CCL20 overexpression promoting tumorigenic phenotypes

J Jandova,[1,2,3] J Janda[1,2,3] and JE Sligh[1,2,3] *1 Southern Arizona VA Healthcare System, Tucson, AZ, 2 Arizona Cancer Center, Tucson, AZ and 3 University of Arizona, Tucson, AZ*

mtDNA mutations are common in human cancers. We examined the role of mtDNA mutations in skin cancer by generating the fibroblast cybrid cells harboring a tRNA mutation encoding the mitochondrial gene for arginine. This specific mutation was commonly found in UV-induced hyperkeratotic skin tumors in hairless mice. Microarrays revealed and qPCR confirmed that this mutation resulted in up-regulation of CCL20 in the mutant cells (mtBALB) compared to wild type (mtB6) cells. Based on reported role of CCL20 in tumor progression we examined if the mtBALB haplotype is associated with higher cellular proliferation and enhanced migration compared to mtB6. mtBALB cells demonstrated significantly increased proliferative and migratory abilities than mtB6 cells. Moreover, treatment of both cybrid genotypes with recombinant mouse CCL20 protein (rmCCL20) resulted in enhanced proliferation of mtB6 cells but had no effect on growth of mtBALB cells. To examine the role of CCL20 on migration of cybrids, we conducted transwell migration assay where mtB6 cells were either pretreated with rmCCL20 or rmCCL20 was added to the bottom well as a chemoattractant. mtB6 cells pretreated with rmCCL20 showed a statistically significant increase in migration compared to untreated cells. In experiments where rmCCL20 was used as a chemo-attractant, mtB6 cells showed even more profound increase in migration almost to the level of mtBALB cells. In co-culture experiments, where mtBALB cells over-expressing CCL20 were used as chemotactic stimuli, mtB6 cells migrated toward the stimuli at significantly higher rate than mtB6 cells without the stimuli. NF-κB is a key transcription factor for production of cytokines. An inhibitor of NF-κB activation, Bay11-7082, inhibited the expression of CCL20 in mtBALB cells and also blocked the enhanced motility seen when rmCCL20 was added. Enhanced proliferative and migratory capabilities caused by up-regulated CCL20 may contribute to the malignant phenotypic characteristics of mutant cells.

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Whole-exome sequencing in a single proband reveals a mutation in the CHST8 gene in autosomal recessive peeling skin syndrome

M Kurban,[1] R Cabral,[1] M Wajid,[1] Y Shimomura,[1] L Pethukova[1,2] and AM Christiano[1,3] *1 Dermatology, Columbia University, New York, NY, 2 Epidemiology, Columbia University, New York, NY and 3 Genetics & Development, Columbia University, New York, NY*

Generalized peeling skin syndrome (PSS) is an autosomal recessive genodermatosis characterized by lifelong, continuous shedding of the upper epidermis. Using a combination of whole-genome homozygozity mapping together with whole-exome sequencing, we identified a novel homozygous missense mutation (c.229C>T, R77W) within the CHST8 gene, in a large consanguineous family with non-inflammatory PSS type A. CHST8 encodes a Golgi transmembrane N-acetylgalactosamine-4-O-sulfotransferase (GalNAc4-ST1), which we show to be expressed throughout normal epidermisby immunofluorescence staining. A colorimetric assay for total sulfated glycosaminoglycan (GAG) quantification, comparing human keratinocytes (CCD1106 KERTr) expressing wild type and mutant recombinantGalNAc4-ST1, revealed decreased levels of total sulfated GAGs in cells expressing mutant GalNAc4-ST1, suggesting loss-of-function. Western blotting revealed reduced expression levels of mutant recombinant GalNAc4-ST1 compared to wild type, suggesting that accelerated degradation may result in loss of function, leading to PSS type A. This is the first report describing a mutation as the cause of PSS type A, discovered using autozygosity mapping combined with whole exome sequencing in a single individual.

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Use of an Er: YAG laser to facilitate nucleic acid delivery to skin

RP Hickerson,[1] C Deguara,[2] CH Contag,[3] LM Milstone[4] and RL Kaspar[1,3] *1 TransDerm Inc., Santa Cruz, CA, 2 Sciton, Inc., Palo Alto, CA, 3 Stanford University, Stanford, CA and 4 Yale University, New Haven, CT*

The ability to alter gene expression with nucleic acid-based therapeutics including short interfering RNAs (siRNAs) and triplex forming oligonucleotides (TFOs), has opened up novel treatment opportunities for skin disorders. Translation of these potential therapeutics to the clinic has been hampered, in part, by the lack of efficient delivery systems that allow penetration through the stratum corneum outer barrier. We evaluated the ability of an erbium-doped yttrium aluminum garnet (Er:YAG) laser to facilitate siRNA and TFO delivery with the goal of selective inhibition of target gene expression or introduction of heritable DNA changes, respectively, to treat a variety of genodermatoses. We used the Er:YAG laser to form highly-reproducible arrays of "holes" through the stratum corneum in murine and human skin. As a thin "coagulum" forms during laser ablation (which may hinder siRNA diffusion into the skin), a variety of techniques were investigated to find the optimal method(s) for its disruption or removal. Murine and human skin were treated with the Sciton Er:YAG laser (with or without coagulum removal), and labeled nucleic acid was applied to the resulting grid. Fluorescence microscopy showed penetration of the labeled nucleic acid into the live layers of the epidermis and labeled TFOs were shown to localize to the nuclei indicative of cellular uptake. Delivery was further enhanced by occlusion. The use of the Er:YAG laser has the potential to facilitate delivery of nucleic acid-based therapeutics for previously untreatable genetic skin disorders.

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TRIM32 regulates Th1/17 vs Th2 response to TLR activation, leading to psoriasis-like vs atopic dermatitis-like inflammatory diseases in mice

Y Wang, E Swanzey, J Lagowski, Y Liu and M Kulesz-Martin *Dermatology, Oregon Health & Science University, portland, OR*

The common inflammatory diseases psoriasis and atopic dermatitis (AD) have been linked to imbalances of Th17/1 and Th2. Toll-like receptors (TLRs) such as TLR 7/8 targeted by imiquimod (IMQ), with mediation through NF-κB, are implicated in their pathogenesis. TRIM32, an E3 ubiquitin ligase with roles in innate immunity, is increased in human psoriasis lesions. We studied Trim32-/- (KO) and K14Trim32Tg (Tg) mice to assess the roles of Trim32 in inflammatory diseases. Tg mice exhibited a psoriasis-like phenotype more severe than wild type (WT) upon treatment with IMQ, including thickening of the epidermis, hyperkeratosis, and dermal inflammatory cell infiltrates. In contrast, KO mice showed thickened epidermis with spongiosis, degranulation of mast cells and eosinophils in the dermis reminiscent of AD, with elevation of plasma IgE levels. Cytokines found in human psoriasis including IL23, IL17F, IL1, and IL22, as well as TNFα and IL18 mRNAs, were elevated in Tg mice compared to WT, consistent with Th17 and Th1 cells involvement. The IMQ treated KO mice had significantly reduced levels of Th17 and Th1 cytokines and RelB compared to WT, however levels of Th2 cytokine transcripts for IL4 and IL5, associated with human AD were elevated. The KO mice showed decreased levels of RelA, with increased levels of pIκBα. Further indication of NFκB activation status included increased RelA nuclear localization in Tg and WT skin lesions compared to cytosolic localization in KO mice, consistent with impaired nuclear translocation, p65 and Th1 cytokine response reported in AD in patients [Dieckhoff, 2005]. These results suggest that TRIM32 may have a critical regulatory role in the cytokine balance of T helper type-17/1 (positive regulation) and type-2 responses (negative) to TLR activation, mediated at least in part through NF-κB. This reveals TRIM32 as a novel etiological mechanism and potential target to be explored in human psoriatic and atopic inflammatory diseases.

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Gene correction of Spanish recessive dystrophic epidermolysis bullosa patient-specific induced pluripotent stem cells

N Umegaki,[1] M Itoh,[1] R Murillas,[3] M Del Rio,[3,4] AM Christiano[1,2] and F Larcher[3] *1 Dermatology, Columbia University, New York, NY, 2 Genetics and Development, Columbia University, New York, NY, 3 Epithelial Biomedicine Division, CIEMAT-CIBERER, Madrid, Spain and 4 Bioengineering, CIEMAT-CIBERER, Madrid, Spain*

Epidermolysis bullosa (EB) is a group of inherited skin disorders characterized by blistering and scarring. The recessive dystrophic form of EB (RDEB) is caused by mutations in the gene encoding type VII collagen (COL7A1) which is the exclusive component of the anchoring fibrils the basement membrane zone (BMZ) of the dermal-epidermal junction. Currently, there is no specific therapy available for patients with RDEB. The potential therapeutic value of induced pluripotent stem cells (iPSCs) has been recently demonstrated from patients with various diseases. We aim to apply this technology to RDEB patients from Spain. Mutational analysis of COL7A1 in a large Spanish cohort revealed that the pathogenic mutation c.6527insC in exon 80 accounted for 46.3% of all Spanish RDEB alleles. All patients with this premature termination codon clinically develop severe generalized blisters and scar formation. Here, we present our efforts toward developing gene correction using patient-specific induced iPSCs (PS-iPSCs) that are homozygous for the highly recurrent RDEB Spanish COL7A1 mutation. First, PS-iPSCs were generated from skin fibroblasts taken from Spanish patients by retroviral transduction with Oct3/4, SOX-2, MYC and KLF4. We confirmed the authenticity of these iPSCs using gene expression analysis by RT-PCR and immunostaining for stem cell markers. Using a COL7A1 exon 80 targeting vector, gene correction of the mutation c.6527insC in PS-iPSCs is underway. The potential of generating functional keratinocytes and fibroblasts from gene corrected PS-iPSCs could provide a vast benefit for the large population of RDEB patients in Spain who carry this recurrent mutation.

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Long-term follow-up after cultured epidermal autograft in a recessive dystrophic epidermolysis bullosa patient

S Shinkuma,[1] D Sawamura,[1,2] H Kawasaki,[1] H Nakamura,[1] M Inoie,[3] W Nishie[1] and H Shimizu[1] *1 Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan, 2 Dermatology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan and 3 Japan Tissue Engineering Co., Ltd, Gamagori, Japan*

Recessive dystrophic epidermolysis bullosa (RDEB) is characterized by mucocutaneous blistering disease. Skin ulcers occurred in RDEB patients are sometimes persistent and intractable despite various treatments, which occasionally leads to squamous cell carcinoma. It has been reported in a small number of RDEB cases that refractory ulcers were successfully treated with cultured epidermal autograft (CEA). There is, however, still controversy as to whether the process of isolating and culturing keratinocytes may induce genetic modifications or enhance cell stem properties, potentially generating an increased risk of tumorigenesis. So far, a long-term follow-up study in RDEB treated with CEA has never been reported. To document an RDEB patient transplanted with CEA for long period and to evaluate the safety of CEA treatment. The patient was a 12-year-old boy with RDEB, generalized other. The CEA was prepared from keratinocytes taken from his dorsum, which had not been involved in. After sterilization, the CEA were applied to the wounds area and fixed with the bandage. Three days after CEA procedure, spotted engraftments were observed and almost all re-epithelialized one month after. Ten years later, the scar is observed at the grafted area and the flexibility and texture of the lesion is similar to the other scars, which have epithelialized spontaneously. The result indicate that there have been no evidence of tumorigenesis after CEA derived from RDEB patient during long-term observation and CEA could be a potential treatment modality in RDEB patients.

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Residual genomic signature of atopic dermatitis despite clinical resolution with narrow band UVB

JK Gittler,[1,2] M Suarez-Farinas,[2] A Shemer,[3] I Cardinale,[2] JG Krueger[2] and E Guttman-Yassky[1,2] *1 Department of Dermatology, Mount Sinai School of Medicine, New York, NY, 2 Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY and 3 Department of Dermatology, Tel-Hashomer Hospital and Tel-Aviv University, Tel Aviv, Israel*

Atopic dermatitis (AD) is a non-scarring chronic inflammatory skin disease. Narrow band ultraviolet (NB-UVB) is one of a few therapies that induce clinical resolution of AD. However, the disease residual genomic signature that persists, despite clinical resolution, has not yet been defined. We analyzed the residual genomic and histologic changes, in clinically resolved lesions of 12 patients with moderate-to-severe AD, treated with NB-UVB for up to 12 weeks, as defined by changes in the AD transcriptome (the differentially expressed genes between lesional and non-lesional (NL) skin), before and after treatment. Genes with <75% improvement were defined as the residual genomic disease phenotype (RGDP). 115 upregulated and 243 downregulated probe sets (73 upregulated and 171 downregulated genes) from the AD transcriptome were part of the RDGP. Many lipid metabolism and structural genes such as PPAR-gamma, leptin, aquaporin-7, lipoprotein lipase (LPL), and Claudin 8, and inflammatory genes, such as CCR1, CXCL2, Angiopoietin-1, CCR1 and CCR7, which function in leukocyte chemotaxis, did not return to NL levels. Although most inflammatory cell markers improved, several DC markers (i.e. FcERI+ and CD11c+) did not resolve. Our novel method determined that although infiltrating T-cells are appropriately eliminated, clinically resolved lesions still express chemotactic and inflammatory DC molecules. Key players in lipid metabolism and structural genes also show residual molecular alterations. Although some of our findings might be specific to NB-UVB treatment, since AD lesions tend to recur after treatment, usually in sites of prior involvement, this gene set has important implications for analysis of progressive alterations with novel therapeutics.

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Alopecia Areata genome-wide association study

L Petukhova,[1,2] S Ripke,[4,5] T Becker,[6,7] M Duvic,[8] M Hordinsky,[10] D Norris,[11] V Price,[12] J Mackay-Wiggan,[1] S Redler,[13] WV Chen,[9] CI Amos,[9] A Lee,[14] PK Gregersen,[14] B Blaumeiser,[15] D Altshuler,[4,5] MJ Daly,[4,5] M Nöthen,[13] R Betz[13] and AM Christiano[1,3] *1 Dermatology, Columbia University, New York, NY, 2 Epidemiology, Columbia University, New York, NY, 3 Genetics & Development, Columbia University, New York, NY, 4 Analytic and Translational Genetics Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 5 Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, 6 Institute for Medical Biometry, Informatics and Epidemiology, Univ of Bonn, Bonn, Germany, 7 German Center for Neurodegenerative Diseases, Bonn, Germany, 8 Dermatology, MD Anderson Cancer Center, Houston, TX, 9 Epidemiology, MD Anderson Cancer Center, Houston, TX, 10 Dermatology, U of Minnesota, Minneapolis, MN, 11 Dermatology, U of Colorado, Denver, CO, 12 Dermatology, UCSF, SanFrancisco, CA, 13 Institute of Human Genetics, Univ of Bonn, Bonn, Germany, 14 Feinstein Institute for Medical Research, North Shore LIJHS, Manhasset, NY and 15 Department of Medical Genetics, Univ of Antwerp, Antwerp, Belgium*

Alopecia Areata (AA) is a highly prevalent autoimmune disease in which the hair follicle is aberrantly attacked. An initial genome-wide association study (GWAS) in AA of 1054 cases and 3255 controls could identify 222 SNPs with significant association ($p \leq 5x10\text{-}8$). To expand gene discovery, we performed a joint analysis with an independent GWAS of 1435 cases and 2032 controls. Genotypes were imputed for each data set yielding 1.2 million SNPs. Standard association analysis with logistic regression including PC covariates was performed within each cohort and results were combined with standard-error weighted meta-analysis. All 8 of our previously identified regions again demonstrated association, and seven additional regions exceeded our significance threshold, implicating a total of 15 candidate regions, including some that have not been previously associated with any disease. This work greatly expands our understanding of the genetic architecture of this highly prevalent autoimmune disease.

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RNA profiling of gene expression in pachyonychia congenita skin lesions reveals dramatic changes in microRNA and mRNA expression patterns

RL Kaspar,[1,2] LM Milstone,[3] J Tang,[2] A Vermeulen,[4] KG Sullivan,[4] M Bessette,[4] D Leake,[4] M Schwartz[5] and R Hickerson[1] *1 TransDerm, Inc., Santa Cruz, CA, 2 Stanford University, Stanford, CA, 3 Yale University, New Haven, CT, 4 Thermo Fisher Scientific, Dharmacon Products, Lafayette, CO and 5 Pachyonychia Congenita Project, Salt Lake City, UT*

RNA profiling, including miRNA and whole genome analysis, has the potential to rapidly reveal pathogenic mechanisms (and therapeutic targets) of inherited skin disorders, including the rare skin disorder pachyonychia congenita (PC). PC is caused by mutations, often single nt changes, in genes encoding the inducible keratins 6a, 6b, 16 and 17. In this study, we profiled miRNA and mRNA global expression patterns in PC plantar biopsies (affected and non-affected regions), control skin and human keratinocyte lines. As expected, PC keratin mRNA (both mutant and wildtype) was upregulated ~10-fold in diseased skin. Interestingly, other genes were more highly overexpressed in diseased skin, some up to 100-fold. MiRNA array analysis revealed major differences in miRNA levels, ranging from 4-fold induction to 14-fold reduction in diseased skin, suggesting targets for intervention. Little is known about the complex RNA expression patterns in exquisitely painful PC lesions. For example, although keratoderma and blistering exist in many other skin disorders, little or no pain is present. The results from this study may shed light on the intense pain associated with PC, including potential targets and strategies for pain relief. Furthermore, RNA profiling may be a useful molecular endpoint for clinical evaluation of PC therapeutics, including siRNAs. A small phase 1b PC clinical trial was recently completed (Leachman et al., 2010, Mol. Therapy), using the first siRNA (TD101) in skin and the first to target a mutant gene, with encouraging results. Although PC is a rare disease, the nature of the disorder makes it an ideal prototype skin disorder (known mutations with expression in defined areas), and the lessons learned should be readily applicable to other skin disorders, rare or common.

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Developing a stem cell-based therapy for epidermolysis bullosa simplex by promoting an allele-specific knockout of mutant keratin 14 in induced pluripotent stem cells

G Bilousova, J Chen, M Yasuda and DR Roop *Department of Dermatology, Charles C. Gates Center for Regenerative Medicine and Stem Cell Biology, University of Colorado Anschutz Medical Campus, Aurora, CO*

Epidermolysis bullosa simplex (EBS) is an autosomal dominant disease characterized by basal keratinocyte fragility due to mutations in either keratin 14 (K14) or K5. Although current therapy for EBS is limited to wound care, recent advances in reprogramming somatic cells into induced pluripotent stem cells (iPSC) offer the possibility of developing new approaches for the treatment of EBS. Toward this goal, we have developed a genome editing strategy for EBS patient-specific iPSC using zinc-finger nucleases (ZFN). We have established iPSC lines from an EBS patient carrying a known hotspot mutation (R125C) in K14 (EBS-iPSC) and from a healthy individual (wt-iPSC). The pluripotency of the iPSC lines was confirmed by the expression of embryonic stem cell markers and the ability to form teratomas. The differentiation of wt-iPSCs and EBS-iPSCs into keratinocytes (wt-iPSC-KC and EBS-iPSC-KC respectively) was performed through exposure to retinoic acid and bone-morphogenetic protein-4. Upon derivation, wt-iPSC-KC and EBS-iPSC-KC expressed K14 and K5, and were able to differentiate in response to calcium. However, while wt-iPSC-KC continued to proliferate indistinguishably from normal keratinocytes, EBS-iPSC-KC exhibited a low growth rate and stopped proliferating at passage 3, presumably due to the negative impact of mutant K14. To correct EBS-iPSC, we used a ZFN-based approach designed to introduce a knockout (KO) deletion of the mutant K14 allele, and to leave the wild-type K14 allele intact and functional. Following the delivery of ZFN, we obtained a mixed pool of EBS-iPSC which contained a number of cells with the KO deletion of the mutant K14 allele. We are currently selecting for these mutant K14 KO EBS-iPSC and will determine whether keratinocytes derived from these KO EBS-iPSC can be used for EBS therapy by employing a mouse xenograft model.

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Real-time non-invasive imaging of siRNA delivery to skin by soluble microneedles

TJ Speaker, RH Hickerson, MA Flores and RL Kaspar *TransDerm Inc., Santa Cruz, CA*

Real-time non-invasive imaging by confocal fluorescent microscopy permits observation of fluorescently-tagged siRNA delivery and diffusion from loaded water-soluble polyvinyl alcohol microneedle arrays. We have recently published work showing that microneedles containing self-delivery siRNAs can effectively inhibit target gene expression in mouse and human skin models. In the present study, we utilize a Lucid VivaScope™ imaging system, modified to allow fluorescence detection, to visualize within live skin the transient polymer gel depots resulting from microneedle administration. Use of a fluorescently-tagged siRNA payload affords direct visualization of the nucleic acid active ingredient distribution and diffusion from the administration site. The capacity for complementary visualization of both depot and drug afforded by this technique opens new doors in efforts to optimize delivery systems for skin-based therapeutics.

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Data regarding nails from 101 patients with pachyonychia congenita

P Holler,[1] C Urban[1] and A Rubin[1,2] *1 Dermatology, University of Pennsylvania, Philadelphia, PA and 2 Pachyonychia congenita project, Salt Lake City, UT*

Pachyonychia Congenita (PC) is an ultra-rare genetic disease caused by mutations of the keratin 6a-c, 16, and 17 genes leading to changes in the skin, hair, and nails. To date, there is a paucity of data detailing nail changes associated with PC, and there is little information to guide treatment of the nail abnormalities in PC patients. By focusing on nail findings, quality of life measures, and treatments, we sought to determine how PC patients are affected by nail abnormalities and to identify successful ways they have treated and managed these changes. Survey questionnaire data was collected from a group of 101 genetically-confirmed patients in the International Pachyonychia Congenita Research Registry. Changes in fingernails and toenails were reported at birth in more than one third of patients. A combination of color, shape, and thickness were the most common initial nail changes and yellow and brown the most common nail colors reported. Clippers and sanders were the most common methods used for nail maintenance and clippers had the most reported side effects. Nearly three-fourths of patients reported having nail infections. A high percentage of patients reported feeling embarrassed, self-conscious, and bothered by the appearance of their nails. Podiatrists are the health care providers most commonly see by patients for nail care. From these data, we conclude that the nail changes in PC adversely affect the quality of life of patients both physically and emotionally and further clinical trials are warranted to explore the efficacy of treatments currently used by patients.

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Cross-cell-type analysis of expression profiles towards the understanding of cutaneous immunity

B Singh,[1,2] S Plaisier,[1] P Sieling,[1] RL Modlin[3] and DJ Lee[1] *1 Dirks/Dougherty Laboratory for Cancer Research, John Wayne Cancer Institute, Santa Monica, CA, 2 Boston University School of Medicine, Boston, MA and 3 UCLA School of Medicine, Los Angeles, CA*

Rank-Rank Hypergeometric Overlap (RRHO) analysis finds statistically significant overlap between two independent high-throughput gene-expression profiles. This method allows us to generate hypotheses about novel pathways shared between biological processes. Bacillus Calmette Guerin (BCG) is a vaccine used to protect household contacts against leprosy, a mycobacterial skin infection. To investigate genes induced by BCG that may be relevant for improved cutaneous host defense, we compared gene expression profiles of lesional skin from patients with restricted (tuberculoid, T-lep) vs. disseminated infection (lepromatous, L-lep) to expression profiles from media vs. BCG-treated PBMCs of subjects previously vaccinated with BCG. We observed that BCG-down regulated genes have significant overlap with those expressed higher in L-lep vs. T-lep lesions (n = 137, max log hypergeometric p-value = 68). Functional groups analysis of this set revealed enrichment for the lysosomal pathway ($p = 1.5 \times 10^{-14}$) indicating that increased expression of lysosomal-associated genes (higher in L-lep) correlate with an unfavorable cutaneous host immune response, and suggesting that BCG may induce a beneficial down regulation of lysosomal genes in macrophages (MΦ). Since MΦ are implicated in anti-mycobacterial defense, we further conducted RRHO analysis to compare genes differentially expressed between M1 and M2 MΦ with leprosy profiles. RRHO analysis showed overlap of genes between M2 MΦ and L-lep lesions (n = 1559, max log hypergeometric p-value = 196), also with enrichment for the lysosomal pathway ($p = 9.8 \times 10^{-25}$). Moreover, we found that M2 MΦ have greater proteolytic activity than M1 MΦ. Finally, M2 MΦ treated with BCG exhibit >30% reduced proteolysis compared to untreated M2 MΦ, supporting our RRHO analysis. Our data strongly suggest that BCG may enhance anti-mycobacterial immunity by decreasing lysosomal activity in macrophages.

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ZNF750 is a p63 target that induces KLF4 to drive epidermal differentiation

GL Sen,[1,2] L Boxer,[1] D Webster,[1] R Bussat,[1] K Qu,[1] B Zarnegar,[1] D Johnston,[1] Z Siprashvili[1] and PA Khavari[1] *1 Stanford University, Stanford, CA and 2 UCSD, San Diego, CA*

In a search for dominant mediators of epidermal differentiation, we defined a new essential role for ZNF750. The ZNF750 gene, which has been recently linked genetically to an autosomal disorder resembling sebopsoriasis as well as psoriasis vulgaris itself, encodes a 723 amino acid protein of unknown function that was increased >300 fold during epidermal differentiation. In tissue, ZNF750 was required for normal induction of late differentiation genes mutated in numerous human skin diseases, including FLG, LOR, LCE3B, ALOXE3, and SPINK5. By itself, ZNF750 directly induced progenitor differentiation via an evolutionarily conserved C2H2 zinc finger motif and alanine substitution at any of 4 amino acid residues in this motif abolished ZNF750 capacity to induce differentiation. The epidermal master fate regulator, p63, directly bound the ZNF750 promoter, as verified both by p63 ChIP-Seq and ChIP-PCR of differentiating keratinocytes, and was necessary for its induction. Moreover, ZNF750 restored differentiation to p63-deficient tissue, confirming that it acts downstream of p63 and is both necessary and sufficient for induction of a large subset of p63 differentiation targets. A search for functionally important ZNF750 targets identified KLF4, a transcription factor that activates late epidermal differentiation, with KLF4 genomic binding sequences enriched in the ZNF750-dependent gene set [629 out of 1978 genes (31.8%) with an E-value=1.1x10-705]. Consistent with a ZNF750-KLF4 functional linkage, the gene expression signature of KLF4i and ZNF750i cells during differentiation strongly overlapped with each other [p<10-300]. Mechanistically, this was found to occur by ZNF750 binding to the KLF4 gene at multiple sites flanking the transcriptional start site to induce KLF4 expression. ZNF750 thus directly links a tissue-specifying factor, p63, to an effector of terminal differentiation, KLF4, and represents a potential target for disorders of this process.